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72 **BOOK REVIEWS**

THE YALE JOURNAL
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DANIEL C. DARROW*

Childrens Mercy Hospital, Kansas City, Missouri, and the University of Kansas Medical School, Kansas City, Kansas

THE RÔLE OF THE PATIENT IN CLINICAL RESEARCH OF A PHYSIOLOGICAL PROBLEM**

In this lecture honoring Grover F. Powers I shall try to give some idea of the way our knowledge of disease is advanced in clinical departments. I shall illustrate the methods from my own experience. If I seem to talk only of my own rôle, please remember that such success as I have had was dependent on my numerous associates in the department but particularly on Doctors Powers, Trask, and the men with whom I have worked intimately and shall mention in due time.

As you all know, Dr. Powers was one of the greatest clinicians of his generation. Nevertheless, he never felt that practice of good medicine or the teaching of the best practice was the most important function of a clinical department in a university. He took for granted that this would be done but also knew that excellent practice can be learned elsewhere. He was convinced that a university department should be judged largely by its ability to add to the knowledge on which good practice is based. And he succeeded in making the reputation of the department at Yale, despite its small size, outstanding in this respect. This end was attained largely through his ability to attract excellent young men to the department and create an atmosphere conducive to the study of disease in infants and children.

The practice of medicine starts with the observation of a patient. Clinical research starts in exactly the same way and the same questions are raised. There is no fundamental difference between the practice of medicine and clinical research. If there is a distinction, it lies in the more immediate concern of the practitioner with application of our present knowledge for the benefit of the patient, whereas the clinical investigator also assumes the responsibility of adding to our knowledge of disease. By training and inclination, the investigator tries to answer the questions raised by disease by bringing out new facts and interpretations. Success in clinical research usually requires more knowledge of one of the basic sciences such as physi-

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* Mercy Hospital Professor of Pediatrics, University of Kansas.

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ology, bacteriology, or pathology than is at the command of most practitioners. Although new facts may still be established by conventional clinical examinations, laboratory and experimental observations are usually necessary. The important step is not the type of laboratory examination or experiment, but a train of thought starting from the observations on the patient. If the questions raised merely receive an answer in words that cannot be related to concrete observations, no advance in knowledge is attained. If the questions are so stated that they are answered by controlled observations, new insight into disease develops.

TABLE 1. SERUM ANALYSES IN GASTRIC TETANY FOLLOWING DRAINAGE OF APPENDICEAL ABSCESS ON JUNE 29, 1927

Date	6–30	7–2	7–6	7–8	7–12	7–15
Cl mEq/L	66	74	83	86	98	94
HCO_3 mEq/L	44	41	41	31	25	27
Cation mEq/L	139	137	135	136		
Ca mgm %	9.5	8.4	7.0	7.9	9.6	10.9
pH	7.54	7.57	7.57	7.55	7.49	7.48

Every clinical investigator recalls certain patients who stand out in his mind as the starting point of a train of thought fructifying in controlled observations. I shall show how three patients played such a rôle in research at Yale.

The first patient was seen in the St. Louis Childrens Hospital in 1927. He was a four-year-old boy with severe gastric tetany following an appendiceal abscess. Before coming to the hospital he had been ill for four days with fever, vomiting, and abdominal pain. On admission, he had signs of peritonitis localized to the right lower quadrant. The diagnosis was confirmed when a large appendiceal abscess was drained. The patient continued to vomit almost all fluids for three days and did not take adequate food for eight days. Within 24 hours after the operation, carpopedal spasm and a Chvostek sign indicated tetany. Figure 1 shows the serum concentrations and certain clinical data as I recorded them at the time. It can be seen that chloride is expressed as mg. of sodium chloride and carbon dioxide content as volumes per cent of carbon dioxide. I also penciled in the values in mEq. per liter. Total base was determined at this time instead of sodium and potassium. Table 1 shows the same data in a more legible arrangement.

The evidence of tetany persisted for six days. There were no convulsions. Although serum calcium was slightly low, the tetany may be considered as

due to the profound metabolic alkalosis indicated by the high bicarbonate and pH. The level of total base indicates that sodium concentration was low. Chloride was very low in the initial sample and did not rise rapidly with treatment. Bicarbonate and pH were both very high and responded to treatment even more slowly than the chloride. The patient received hypo-

ST. LOUIS CHILDREN'S HOSPITAL

SURGICAL NO._____

Name_____

BINDING LINE

	6/30/27	7/2/27	7/6/27	7/8/27	7/12/27	7/15/27
Cl. serum	386 66	43374	48683	50386	5.73	550
$H CO_3$ serum	102.8	96406	96406	73.5 31.1	6 0	
Total Base serum	138.5	137	135	136		
PH serum	7.59	7.57	7.57	7.55	7.49	7.48
Ca. serum	9.5	8.4	7.0	7.9	9.6	10.9
P. serum	3.00	3.0	—	—	—	
N.P.N blood	23	—	18	15	23	
sugar blood	132	—	-	101	-	
Lactic blood	10	—	—	—	25	
Protein Serum	563	5.03	5.03	5.67	6.55	
Carpopedal spasm	++++	++	0	0	0	0
Chvostek	++++	++++	++++	0	0	0

FIG. 1.

dermoclyses of physiological saline as long as he vomited and was not able to take adequate fluids. Nevertheless, the metabolic alkalosis and the serum electrolyte concentrations did not return to normal until food was taken.

In 1927, we believed that Gamble[10] had adequately described the pathogenesis of metabolic alkalosis. The disturbance was regarded as due to the loss of hydrochloric acid in gastric secretions. The resulting changes in body composition were considered to be largely, if not entirely, confined to the extracellular fluids. The serum analyses indicated the concentrations in extracellular fluids. The appearance of the patients and the loss of weight were considered to indicate a decrease in the volume of these fluids. Accord-

ingly, the deficit of chloride was equal to the decrease in chloride concentration multiplied by the volume extracellular fluid. The volume of extracellular fluids was uncertain in 1927 and for that matter is still uncertain. Since sodium was regarded as confined to the fluids surrounding the cells, loss of body sodium was considered to be present even though the concentration in serum was normal. According to these concepts, the only deficits susceptible to fluid therapy were losses of water, sodium, and chloride. It was known that normal kidneys could excrete large amounts of bicarbonate and that the excretion of bicarbonate corrects metabolic alkalosis. These patients were known to have essentially normal kidneys and should, therefore, be able to adjust acid-base equilibrium if physiological saline was given in sufficient amounts to provide the sodium necessary for the excretion of bicarbonate.

From the point of view of extracellular fluids, Gamble's description of metabolic alkalosis is still reconcilable with present knowledge. His terminology does not agree with that of modern chemistry. He considered that bicarbonate concentration remained high owing to loss of chloride and, when recovery occurred, that chloride displaced bicarbonate. Descriptively he was correct but from the point of view of modern chemistry, the statements are misleading. Chloride does not react with bicarbonate and therefore cannot directly alter bicarbonate concentration. The fundamental change in metabolic alkalosis is loss of hydrogen and chloride ions from extracellular fluids. It is the hydrogen ions coming from carbonic acid which form bicarbonate and account for the rise in bicarbonate in metabolic alkalosis. Similarly, during recovery hydrogen ions as well as chloride ions increase. It is the hydrogen ions reacting with bicarbonate to form carbonic acid which account for the decrease in bicarbonate. This modern chemical interpretation is not a matter of semantics, but an explanation which brings out the rôle of the hydrogen ions in the acid-base equilibrium of the blood.

Although this patient received abundant physiological saline, the metabolic alkalosis did not respond as the convincing description of Gamble had led us to expect. It was rather generally known that some patients with metabolic alkalosis had acid urines and did not correct the blood changes promptly. Nevertheless, everyone said that metabolic alkalosis leads to an alkaline urine capable of correcting the serum changes if sufficient sodium chloride is available. Everyone was more or less complacent about our understanding of metabolic alkalosis because many, perhaps most, of the patients responded in a manner compatible with Gamble's description of the pathogenesis. I cannot say that I was any less complacent. I merely kept the record in my desk and hoped that some day I would be able to explain the findings satisfactorily. I shall now describe an apparent digression from

the study of metabolic alkalosis occasioned by another case. The studies initiated by this patient eventually prepared me to return to the problem of metabolic alkalosis.

The second patient suffered from a typical attack of infantile diarrhea shortly after I arrived in New Haven in 1928. The balances of water and electrolytes were determined during recovery with the methods available. Figure 2 is taken from a paper written ten years later.[8] This seven-month-old boy had had diarrhea for six days and had vomited a good deal during the last two days. He was regarded as very sick. Skin turgor was greatly

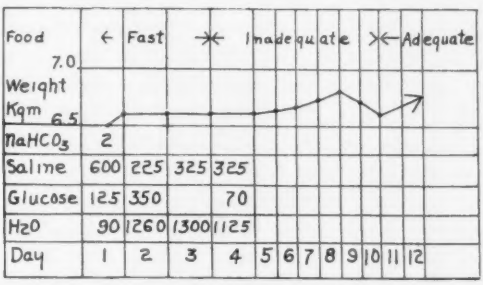

FIG. 2. Infantile diarrhea in a seven-month-old boy. Patient treated as indicated in the chart. All values in grams.

diminished; the eyes and fontanel were sunken, and the abdomen flat. There was Kussmaul breathing. The serum concentrations were: bicarbonate, 14; Cl, 108, and total base, 137 mEq. per liter. In other words, there was evidence of marked dehydration of extracellular fluids with acidosis and low sodium concentration. After three days, the balance data showed a retention of somewhat less than 20 milliequivalents of chloride per kilogram. The balance of total base indicated that about the same amount of sodium was retained. Despite these retentions of sodium and chloride, the practically constant body weight indicated that little water was retained during recovery. These findings showed that recovery from the clinical picture of dehydration could occur without appreciable change in body water.

According to our ideas in 1928, diarrheal dehydration was produced by losses of water, sodium, and chloride in the stools. Emphasis was placed on the losses of sodium and chloride because the losses of potassium which were known to occur were regarded as an accompaniment of starvation and tissue breakdown. The therapeutically important losses were regarded as extracellular water, Na and Cl. It was known that the sodium concentra-

tions of the serum might be low, normal, or high, but little attention was paid to the implications of the sodium concentrations of the serum.

This patient was in the back of my mind when Yannet and I[8] devised a method for the experimental production of losses of sodium and chloride without change in body water. We showed that loss of sodium and chloride leads to low sodium concentrations when body water is constant. However, the blood becomes concentrated with respect to hemoglobin, indicating a decrease in plasma volume. The dogs showed diminished skin turgor, lost their appetites, and did not restore sodium concentration until salt was given. It could be shown that extracellular volume had decreased owing to shift of water into the cells. This is the effect which one would anticipate if osmotic equilibrium is attained between extracellular and intracellular fluids by transfer of water rather than by transfer of cations out of the cells when serum sodium concentration decreases. The data showed that the decrease in Na concentration with a given Na loss is related to total body water. However, the shift of extracellular water into the cells depends on the relative proportions of extracellular and intracellular fluids. Other experiments showed that increase in extracellular sodium concentration produced expansion of extracellular water owing to transfer of intracellular water to the fluid outside of the cells. In part the symptoms depend on the volume of extracellular fluids. To understand the disturbances produced by losses of water and electrolytes, it was necessary to study body composition and define more precisely the distribution of body water and electrolytes. With a reasonably reliable frame of references that depicted body composition, it should be possible to discuss intelligently the changes in body composition.

For this purpose, Harrison, Yannet, and I[11] analysed the bodies of certain animals for water and electrolytes. The data indicated that most but not all chloride is extracellular. Despite some uncertainty, the chloride gave us a first approximation of the volume of extracellular fluids. Sodium was shown not to be confined to the fluids surrounding the cells. About one-fourth was found in the bone salts where it could not be taking an active part in the osmotic properties of these fluids. Considerable amounts of sodium were found in muscle cells and probably existed in other cells as well.

The analyses enabled us to depict body composition as illustrated in Figure 3. The construction is based on the following assumptions: extracellular volume multiplied by the concentration of electrolytes in an ultrafiltrate of serum gives total extracellular electrolytes; intracellular electrolytes are given by the volume of intracellular water multiplied by the probable concentrations in the intracellular water of muscle. Although the assumed values are known approximately, none of the values are precisely correct. Nevertheless, the diagram does serve to illustrate the changes in

distribution of body water and the probable changes in body composition. It has served to point out the critical deficiencies in our knowledge.

Knowing some of the effects of changes in concentration of extracellular electrolytes on the distribution of body water, it was necessary to know what changes develop in the electrolytes within the cells. Miller and I[18] determined the muscle composition in rats subjected to experiments de-

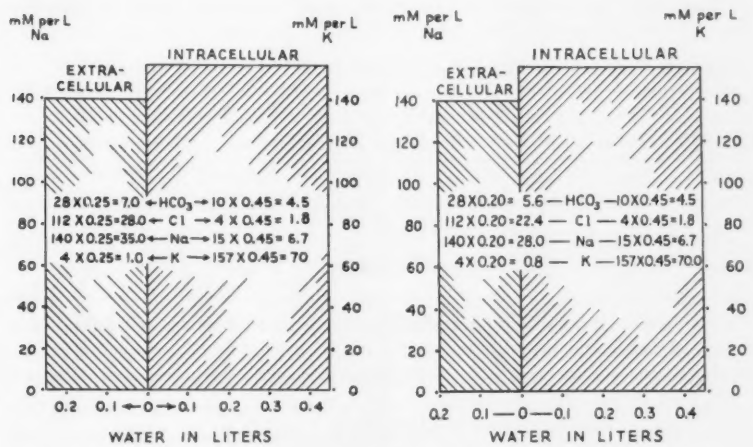

FIG. 3. Frame of reference for depicting body water and the principal electrolytes dissolved in body water. The estimated amounts are indicated per kg of infant on the left and per kg of an adult on the right. Water within the cells is probably nearer 0.4 liter than 0.45.

signed to modify cell composition. Accompanying diets low in potassium and injections of desoxycorticosterone acetate, muscle potassium decreased by as much as one-third of the normal content while intracellular sodium replaced about two-thirds of this deficit. Although our work was done independently, Heppel[19] demonstrated the same findings in young rats fed a diet low in potassium before our work was published. If we had determined serum bicarbonate concentration or had paid attention to the low chloride in the presence of normal sodium, we would have known that we had produced metabolic alkalosis. In other experiments, intracellular potassium increased promptly following the rise in serum potassium resulting from intraperitoneal injections of potassium chloride. However, the potassium left the cells as serum potassium returned to normal. In rats with cells depleted of potassium, injections of potassium chloride produced large and rapid increases in muscle potassium with only a slight rise in serum potas-

sium. It was now clear that the muscles are not subject to significant increases in potassium unless serum potassium is distinctly high but large decreases occur when the output exceeds the intake. This latter change in composition was shown to occur without gross loss of muscle contraction.

These findings completely altered the concept of the equilibrium between extracellular and intracellular fluids that had dominated clinical medicine. The membranes had been regarded as relatively impermeable to cations and consequently sodium could not enter the cells. At present it seems clear that

TABLE 2. BALANCE STUDIES DURING INCREASE AND DECREASE OF ALKALOSIS

Period	Serum HCO_3	Conc. Cl	mEq per L Na		Balance mEq Na	K	K*	Cl
1A	35	61	138	Total	—16	—62		—32
1B	47	55	124	Cell	+50	—60	—69	
3A	45	51	129	Total	+97	+77		+86
3B	35	69	138	Cell	—40	+71	+54	

Period 1, milk mixture only for three days. Dehydration and metabolic alkalosis increased during this period.

Period 2, milk mixture with added KCl. Saline hypodermoclyses given daily. Recovering from dehydration and alkalosis.

K* balance corrected for N balance (1 gm N for 2 mEq K).

the membranes are more or less freely permeable to sodium and potassium. The unequal distribution of these ions on the two sides of the membranes is difficult to explain but it is known that maintenance of cell composition involves active metabolism and normal cellular structures. The work with Miller, Yannet, and Harrison prepared us to expect changes in intracellular potassium when disturbances of body water and electrolytes occur.

About this time, the third case was studied. This patient came to the hospital with a story of diarrhea since birth. Without analysing the serum for electrolytes the patient was treated as a case of infantile diarrhea. Recovery from dehydration seemed satisfactory the next day. Fortunately, I visited Boston the following day and heard Gamble discuss the data accumulated over a period of a year on his case of congenital alkalosis with diarrhea. On returning to New Haven, my patient had again become dehydrated owing to diarrhea, and the serum analyses showed extremely high bicarbonate and extremely low chloride. There had been no vomiting. It was obvious that my patient was like Dr. Gamble's and I could study my case with the benefit of his findings.

The characteristic features of these patients[4,6] were marked metabolic alkalosis and the passage of voluminous watery stools since birth. The stools contained more chloride than sodium and considerable potassium. Serum bicarbonate was always high and chloride, always low; serum potassium was often low. The urine was always essentially free of chloride. Protein fat, and carbohydrate were well absorbed despite the continuous watery stools. The alkalosis could not be corrected with sodium chloride; ammonium chloride produced only a temporary fall in bicarbonate. Potassium was somewhat more successful in restoring serum bicarbonate and chloride concentrations. We know now that I did not give large enough doses of this salt during these studies.

Table 2 shows the balances for two periods of three days and the serum concentrations at the beginning and end of these periods. In period 1 the patient developed the clinical picture of dehydration, and the alkalosis became more marked. In period 3 the general condition improved, and the alkalosis decreased. During this study hypodermoclyses of saline were given each day, and potassium chloride was added to the milk mixture. The table also shows the cellular balances.

In order to estimate the cellular changes in sodium and potassium, the following assumptions[4,6] were made: (i) that extracellular water was 25 per cent of the body weight when dehydration was not present, (ii) that the extracellular concentrations of electrolytes were those of an ultrafiltrate of serum, and (iii) that the balances of chloride reflect changes of chloride in extracellular fluids only. While it is known that these assumptions are likely to lead to errors when the balances are small, the predictions are fairly reliable when the balances are large as was the case in these studies. The calculations based on these assumptions are shown in the following equations:

$$[Cl]_e\,(H_2O)_e = (Cl)_e$$
$$(Cl)_e + (\text{Balance } Cl)_e = (Cl)_{e2}$$
$$\frac{(Cl)_{e2}}{[Cl]_{e2}} = (HO)_{e2}$$
$$(HO)_e[Na]_e = (Na)_e$$
$$(Na)_e - (Na)_{e2} = (\text{Balance } Na)_e$$

The brackets [ ] refer to concentrations and the parentheses ( ) to total amounts. The subscript e refers to extracellular and 2 to the end of the balance period. The cellular balance is obtained by subtracting the extracellular balance from total balance.

During period 1 the calculated decrease in extracellular volume was 380 grams despite a loss of body weight of only 90 grams. The appearance of

the patient indicated a considerable loss of extracellular water. Fifty milliequivalents of sodium entered the cells while 60 mEq of potassium left the cells. The deficit developed in the cells would be 69 mEq if the balance of nitrogen is taken into account. In other words, increasing metabolic alkalosis was accompanied by increase in intracellular sodium and decrease in potassium. In period 3 metabolic alkalosis decreased while intracellular sodium decreased 40 mEq and potassium increased 71 mEq. The change in potassium in relation to nitrogen balance would be 54 mEq. Body weight increased 240 grams while the calculated extracellular volume increased 920 grams. Attention is directed to the fact that over-all balances do not indicate the shifts of sodium and potassium between intracellular and extracellular fluids. Thus the calculations developed in order to interpret the balances in this patient provided a method of interpreting data in other patients as well as giving new insight into metabolic alkalosis.

At this time we knew a good deal about the changes in composition of muscle in rats. We knew that similar changes occur in patients. In 1945, a paper discussed the probable relation of changes in intracellular composition to disturbances in body water and electrolytes in patients.[5] It could be confidently predicted that considerable losses of cell potassium would be found in both metabolic alkalosis and acidosis, i.e., in alkalosis due to loss of gastric secretions and acidosis due to diarrhea, diabetic acidosis, and probably many other disturbances. It was known that the changes in cell composition would respond to administration of potassium. It was this patient with congenital alkalosis with diarrhea who made me certain that metabolic alkalosis is accompanied by loss of muscle potassium and retention of sodium in the cells. However, the details of the pathogenesis of metabolic alkalosis were not apparent. It was this patient who made me confident that the administration of potassium to infants with diarrhea would be beneficial if the deficits of potassium were as great as I expected.

The study by Schwartz, Iannucci, Coville, and myself[7] brought the relationship of cell composition to acid-base equilibrium clearly into view. Both metabolic acidosis and alkalosis were produced by inducing a deficit of one of the ions, sodium, chloride, or potassium. The rats were permitted to adjust to the deficit of one ion in the presence of abundant intakes of the other two ions. Under the conditions of these experiments serum bicarbonate concentration varied directly with intracellular sodium of muscles and inversely with potassium. Deficit of chloride accompanied by metabolic alkalosis resulted in the same changes in muscle composition as were produced by primary deficit of potassium. Likewise, primary deficit of potassium produced the same changes in serum concentrations, namely, decrease

in chloride and high bicarbonate. It was now established that metabolic alkalosis produces loss of cell potassium, and loss of cell potassium produces metabolic alkalosis. The experiments did not reveal whether this relationship was dependent on the equilibrium between extracellular and intracellular fluids or a change in renal function.

At this point Cooke, Segar, Cheek, and a number of students clarified this point.[1] It is the effect of metabolic alkalosis on renal function which leads to deficits of potassium; it is the deficit of potassium that alters renal function so that metabolic alkalosis develops and is maintained. The evidence can only be briefly summarized in this lecture. Prolonged large intakes of sodium bicarbonate do not produce metabolic alkalosis unless the intake of potassium chloride is very low. However, the intake of potassium is more important in preserving the renal defense against alkalosis since a high intake of potassium preserves renal defense against quite low chloride intake, while chloride does not preserve renal defense when the intake of potassium is low. The rats did not develop alkalosis without decrease in cell potassium and increase in intracellular sodium.

The data bring out clearly that rats with metabolic alkalosis and potassium deficiency have an excess of sodium in the cells. Probably there is little or no deficit of chloride in the body as a whole despite the low serum concentrations. Thus the electrolyte deficits are chiefly, if not solely, potassium. It should not, therefore, be too surpising that the changes in body composition can be corrected with either potassium chloride or potassium bicarbonate without any external source of sodium chloride. The changes in muscle and the balances of electrolytes on giving these potassium salts reveal two mechanisms involved in recovery from metabolic alkalosis. The first is the exchange of extracellular potassium for intracellular sodium and hydrogen. The second is the excretion of bicarbonate with potassium as well as sodium. Both mechanisms require replacement of potassium.

The exchange between extracellular fluid and muscle may be depicted in three stages when KCl is given: (i) 3 KCl are added to extracellular fluids, (ii) 3 K enter the cell in exchange for 2 Na and I H, (iii) this leaves extracellular fluids with 2 NaCl and 1 HCl. The HCl reacts with Na HCO_3 to form NaCl. Serum bicarbonate is reduced by hydrogen ions derived from the cells and the kidneys play no rôle in the recovery from alkalosis.

When $KHCO_3$ is given, the exchange is the same: (i) 3 $KHCO_3$ are added to extracellular fluids, (ii) 3 K exchange for 2 Na and 1 H, (iii) extracellular fluid then has 2 $NaHCO_3$ and 1 H_2CO_3. Bicarbonate is increased, and restoration of serum bicarbonate concentration is attained by excretion of base in the urine which is equivalent to retention of hydrogen ions. We

were surprised that the base excreted was not largely bicarbonate but organic anions.

In both types of experiments, sodium was lost from the muscles as potassium was retained. The changes in muscle composition in metabolic alkalosis indicate an excess of intracellular hydrogen ions which are released during recovery. Indeed the increase in cellular protons is greater than the decrease in extracellular fluids. The experiments give no evidence that the body as a whole suffers a deficit of hydrogen ions in metabolic alkalosis with potassium deficiency. Consequently the administration of ammonium chloride is not indicated. This salt can merely temporarily decrease extracellular bicarbonate while aggravating the disturbance in hydrogen ions in the body as a whole. There is evidence that the deficit of chloride ions may be negligible or at least much smaller than the decrease in extracellular concentration would lead one to expect. The rats receiving potassium bicarbonate restored serum chloride concentration without an external source of chloride. The rats lost considerable weight. Probably total body chloride was essentially normal owing to expansion of extracellular volume. With treatment, the contraction of extracellular water increased serum chloride concentrations to normal.

The pathogenesis of metabolic alkalosis was now reasonably clear. Renal defense against alkalosis leads to increased potassium excretion as long as potassium is available. The defense is successful as long as muscle potassium can be maintained. When the cells become deficient in potassium, renal function is altered. The kidneys now fail to maintain normal serum bicarbonate and chloride concentrations; the concentrations characteristic of metabolic alkalosis now develop and are maintained despite abundant excretion of sodium and chloride. The changes in muscle composition indicate that about two-thirds of the deficit of potassium is replaced by sodium. The cellular deficit of cations is apparently replaced by hydrogen ions. This paradoxical increase in hydrogen ions in the cells while there is a decrease of these ions in extracellular fluids is not satisfactorily explained. Nevertheless, it is evident that hydrogen ions are donated to extracellular fluids as cell composition is restored.

It is probable that aldosterone and other corticosteroids that increase urinary potassium play a rôle in the pathogenesis of metabolic alkalosis. Metabolic alkalosis develops in some types of adrenal hypercorticalism owing to losses of potassium. Activation of the adrenal cortex by operations has been shown to occur and probably aggravates the potassium losses. The administration of a number of corticosteroids produces metabolic alkalosis.

We are now in a position to explain case 1. The patient developed metabolic alkalosis owing to loss of hydrochloric acid in vomitus. Consequently, urinary excretion of potassium increased and deficits of this ion developed when food could not be taken in sufficient amounts to replace the losses. Under these circumstances, administration of large amounts of sodium chloride failed to correct the alkalosis. There is evidence that, if the urine contains large amounts of sodium, potassium excretion increases. The patient finally recovered when he took enough food to restore the deficits of potassium.

This patient is typical of many cases of metabolic alkalosis formally seen on the surgical wards, especially in those subjected to gastric suction after operations. The recognition of the relation between potassium deficiency and alkalosis has enabled the surgeons to provide appropriate fluids after operations. It has permitted them to succeed with operations that were all but impossible before potassium was administered.

I shall now return to my original thesis that clinical research starts with the observations of the patient. As in the present discussion, phenomena may be observed which require physiological explanations. When this is the case, the clinical investigator must use physiological methods. He often opens a new field of physiology as well as clinical medicine. This type of physiological research is not likely to be done by men actively engaged in the practice of medicine. Neither is it likely that these observations will be made by pure physiologists, yet the experiments are essentially physiological. Consequently, some clinical research becomes an essential part of pure physiology.

We are all familiar with the dependence of clinical medicine on physiology. We may sometimes forget that physiology is just as much dependent on sound clinical observations as it was in the past. The answers to the questions raised by disease are directly applicable to physiology. Not only do the observations of clinical research become a part of our knowledge of how the body works, but the techniques of study which at first seem peculiar to clinical medicine often prove the simplest method for observations not directly related to disease. Likewise, I have found that the phenomena of disease are more readily explained by physiological experiments than by direct observations on patients which are always so difficult to control. Nevertheless, the patients, as the present discussion has shown, are essential to orient physiological observations so as to answer the questions raised by disease. Consequently, I have no sympathy with attempts to confine physiological observations to physiological laboratories. As corollary, I have no sympathy with restriction of clinical research to the observations that can be

made on patients. In the long run no sharp distinction between the knowledge of physiology and clinical science can be justified.

In the past thirty years clinical research has become an important feature of the medical schools of this country and has been actively supported by the universities. However, there is danger that the universities may let this field slip from their hands now that clinical research is conducted on such an elaborate scale in institutes. With an eye on budgets, administrators of medical schools and hospitals connected with medical schools may be willing to let this essential function of the medical schools and these hospitals be done elsewhere. The clinical investigator may be regarded as an amphibian who is not well adapted to the environment of pure research or that of clinical medicine. It may be decided that the sole function of the hospital is the care of the patient and the sole function of the medical school is teaching the best of current medical practice. The lack of adaptation of the clinical investigator is only apparent for there is a large region that involves both the clinic and the laboratory where a rich life can be lived in a truly amphibian environment. Exploration of this area is essential to the development of real university clinical departments.

When the Yale University School of Medicine was reorganized over thirty years ago, it was decided that all departments could and should be conducted at the level of graduate education. The medical faculty was given control of the laboratories and the wards in order to study disease as well as to care for patients. It was felt that in no other way could the clinical departments conduct medical education at the graduate level. The idea was to get away from teaching medical practice as a trade. I believe that the important distinction between a trade school and a department of a university is as follows: A trade school is concerned with teaching the best in current practice of a trade while a university is concerned with advancing the knowledge on which the practice of a trade is based. A trade school is busy with indoctrination of the students while a university prepares the students for the future developments of the field.

It is not only the medical schools which recognize this point of view. An industrial engineer told me that it is practically useless to teach solely practice in a field of engineering because the practice becomes obsolete in five years. We all know that this is true in medicine. If a university department in a medical school does not recognize that the study of disease is an essential function, the students are not likely to be prepared for the essential mental processes of medical practice; they will not come in contact with the advanced students of disease and will not be well prepared for objective evaluation of the advances in medical science.

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EARLY PREGNANCY AND FERTILITY TESTS

Many are the waies Authors have left for Women to know whether they be with Child or not, which happen true in many Women, but not in all . . . —Nich. Culpeper[39]

Whether a woman has become pregnant and whether she is able to become so have been urgent questions since mankind began to speculate about the future. Many are the procedures by which men and women have tried to make Nature reply. Most of the "tests" are very old, and almost all of them are unreliable. Whether they originated in the chilly caverns of superstition, were developed empirically, or were devised on a theoretical basis, they exemplify man's search for the answers to important mysteries. A review of the methods discloses some interesting historical patterns.† In such a review it is preferable to consider pregnancy and fertility tests together because, as we shall see, the wording of the prognosis was sometimes ambiguous. Most often it was stated, in effect, that if the test had a certain result, the woman "will bear." Whether this meant that she had conceived, or simply that she could do so, might not be specified.

Uroscopy, diagnosis by examination of the patient's urine, had been practised since the early days of Arabic medicine and possibly before. The urine of pregnancy was believed to have special features, particularly if it were stored in a glass container for a few days. A special characteristic was the pearly film or *Kyesteine* reported to form on the surface. The story of this phenomenon has been well told by Marshall,[58] and will not be repeated here. It appears that the surface layer is due to the stimulation of bacterial growth by the hormones of pregnancy. Curiously enough, although Avicenna, Hippocrates, and Savonarola, according to Marshall, were aware of the changes occurring in stored pregnancy urine, the *Kyesteine* was not fully described until Nauche published his observations in 1831. A century later, Aschheim and Zondek[5] devised their test, now widely used, which, although it does not depend on the appearance of the specimen, is based also

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† I have drawn on many sources, particularly the excellent papers by Iversen[46] and Henriksen[44] and the equally valuable books by Fasbender[20] and Dawson.[24] My purpose is to collect and review material already cited and to add a good deal which is obscure or new.

on a high titer of particular hormones—gonadotrophins—in the urine of early pregnancy.

MacKinney[55] has called attention to an amusing tale which illustrates the skill once attributed to the uroscopist. In the tenth century, Notker Balbus was a famous healer of St. Gall, Switzerland, and was also physician to a certain Duke Henry. To Balbus the Duke gave as his own a urine specimen which actually was that of a pregnant lady of the court. Notker made his inspection and announced that a miracle had come to pass: in thirty days the Duke would be delivered of a son! The Duke had the grace to blush and to reward his crafty physician.[50] Joubert,[47] a later observer, scorned this kind of pregnancy test: "Thus it is that very often these diviners are deceived by being given the urine of a man, whom they say is great with child, which quite properly causes roars of laughter."

Actually, the inspection of urine to determine if a woman will have a child is mentioned in the fascinating Berlin medical papyrus,[12] thought to date from the XIXth or XXth Dynasties (approximately 1350-1100 B.C.); turbid or sedimented urine indicated that she would bear. Avicenna (980-1037 A.D.) spoke of a surface cloud, a yellow iridescent color, a cotton-like mass, and granules as diagnostic signs.[40] Savonarola[74] begins his ponderous discussion, *Urinae praegnantium debent cum magna solemnitate considerari:* "Pregnancy urines should be regarded with great solemnity." He, Rueff,[69] Bonaccivoli,[5] and Castro[15] certainly considered such urines not only solemnly but in enormous detail. John Cotta, writing at about the same time, however, condemned this kind of diagnostic effort by listing it in his book, *A Short Discoverie of the Vnobserved Dangers of several sortes of Ignorant and Vnconsiderate Practices of Physicke in England.*[19]

The surgeon Cornelius Solingen[78] stated that Fernelius (presumably this was Jean Fernel, 1497-1558, professor of medicine at Paris) recommended mixing a suspected urine with wine: "If the liquid becomes turbid, as though beans had been cooked therein, then the woman is pregnant." But, as Aschheim[4] points out, Solingen himself remarked, "That such a thing may be true, I let my geese believe." The same test was also used in what is now Czechoslovakia.[45]

It is a remarkable fact that several of the pregnancy tests which appear in the early medical papyri of the Egyptians can be traced with reasonable certainty into Greek and thence to Roman and sixteenth century medicine. Dawson,[24, 25] Iversen,[46] and others[16, 60, 33, 54, 32, 29, 65] give the details of this progression, not all of which can be presented here. One example is found in Prescription 199 of the great Berlin medical papyrus. It is directed that wheat and spelt (a kind of cereal) be placed in two flasks[58] or cloth packs[58] with dates and sand and watered daily with the woman's urine. If they

sprout, she is pregnant.* Iversen,[48] who offers impressive evidence that several European pregnancy tests can be traced back to the early Egyptian papyri, bases his argument on his translation of the Carlsberg papyrus. He dates this document from the Twelfth Dynasty (about 2000-1800 B.C.) or even earlier. Prescription III of the Carlsberg papyrus is missing in part, but Iversen believes that it agrees with Prescription 199 in the much later Berlin papyrus.

Lüring[54] has pointed to a parallel passage in Galen:

> Make two little holes containing the urine of a pregnant woman; in one throw barley and the other wheat. Then sprinkle with the urine and shovel earth on top. If the grains of wheat sprout first, she will bear a boy, but if the barley seeds, a girl.[50]

A Galenic test for fertility is similar[54]: when a couple is childless, put a lentil in the urine of each. The lentil will sprout in the urine of the fertile, but not in that of the sterile, individual. Lüring[54] added that a method ascribed to Moschion (sixth century A.D.) is also comparable, as it advises watering barley or peas in earthen vessels with the urine of the man and woman; sprouting demonstrates fertility.

The test turns up again in Antonio Guainerio's *De egritudinibus matricis,*[51] one of the very early printed books on a medical subject; the use of beans or barley is suggested. In 1540 appeared *The byrth of Mankynde.*[60] This was Richard Jonas' translation of *Eucharius Rösslin's* famous *Der Swangern frawe*[*n*] *und hebamme*[*n*] *Roszgarten.*[65] This very popular work was the first printed book on midwifery and the first to be devoted entirely to this subject.[67] Rösslin and Jonas gave more details for the test, e.g., to plant the grain in "ii. pottes/such as they set gyly-flowers in/ fill them with good earth" and to continue to water the pot with urine daily for eight or ten days, watching to see which seeds would germinate and prove thereby the fertility of the men and women, "but see that there come no other water or rayne on the pottes." Rueff[60] modified the fertility test.

The appalling *Dreckapotheke* of Paulini,[62] published in 1714, recommended the test, ascribing it to one Peter Beyer. Iversen[48] identifies Beyer or Boyer as a famous sixteenth century physician of Florence. Beyer's sources, Iversen believes, were Bursian (apparently an early Greek medical writer) and, probably, Galen. Iversen does not accept Ebers'[20] theory that the test came into European medical literature via Constantine the African (1020-1087 A.D.) and the Salernitan School, although Constantine did

* Here, as in many other prescriptions, directions are also given for determining whether the child will be boy or girl. I plan to deal with the subject of sex determination in another paper.

describe the test.[10] Erman[32] seems to have been the first to call attention to the repetition in Paulini's *Dreckapotheke* of the method which had originally appeared in the Berlin medical papyrus. Le Page Renouf[68] had also pointed to the close parallel between the method described in the Berlin papyrus and those in *The Experienced Midwife*,[8] a compilation appearing under the pseudonym of Aristotle. It seems certain that this and other pregnancy and fertility tests can be traced back from eighteenth century European medical lore to the great medical papyri of early antiquity.

Henriksen[44] reminds us that recent studies indicate that the hormones which are present in pregnancy urine may indeed stimulate the germination of seeds and the growth of plants. The evidence is controversial but important. In the first place, there are "plant hormones" called auxins which accelerate plant growth and development. Curiously, one or more of the auxins is excreted in human urine; possibly they are ingested in vegetable foods.[49, 11] Recently, some doubt has been expressed as to whether auxins, potent though they are, actually originate in plants. Indoleacetic acid, isolated from urine as well as from plants, may also be a growth factor; certainly it can stimulate the growth of oat coleoptiles.[9] In summary, then, human urine may contain plant hormones which stimulate plant growth, but the level of these hormones does not appear to increase in pregnancy. This does not support the Egyptian pregnancy test but is of interest in connection with the test for fertility.

In the second place, hormones of *animal* origin may also stimulate plant growth. Crystalline ovarian follicular hormone had no effect on oat seedlings,[49] but crude aqueous extracts of ground calf ovaries did speed the growth of black mustard and yellow lupine seedlings and of stems of forsythia, plum, wall flower, lilac, and garden sage as compared to controls.[79] The ovarian hormones estrone and estradiol and some hypophyseal hormones definitely stimulated the growth of corn, onions, a meadow grass (*Poa*), fuchsia, the Reine marguerite, and hyacinths; and estrone accelerated bud opening of hazel, forsythia, and horse chestnut.[75, 17, 83, 84, 13] The significance of all this is that these hormones also occur in pregnancy urine. Finally, Henriksen[44] reports that when he tested the ability, as prescribed in the Berlin papyrus, of 50 urines to germinate seeds as a pregnancy test there were 75 per cent correct positive, and 85 per cent correct negative, tests. Thus the possibility of some empirical basis for the pregnancy and fertility tests involving plant growth is not excluded.

The ability of urine to damage some plants also was the basis for a fertility test. In a relatively recent (third century A.D.) papyrus discovered at Thebes,[30] it is directed that the woman should urinate on an (unspecified) plant in the evening. If next morning the plant is scorched, she will

not become pregnant, but if the plant is undamaged, conception is possible. Dawson[26] and Iversen[40] regard this method as a variant of the pregnancy test already described. Iversen[40] points out that a very similar passage occurs in the writings of Avicenna; here the recommended plant is lettuce, and Iversen shrewdly relates the Egyptian hieroglyph for *scorched* and Avicenna's *exsiccaverit*. Rueff[69] suggests poplars, nettles, beans, or barley as plants that may be used. Iversen[40] refers to appearance of the test in Ryff's* *Frauen Rosengarten*[71, 73] (poplars, nettles, beans, or barley), Kunrath's *Medulla Destillatoria* (wild poplars),[51] and Culpeper's translation of Lazarus Riverius (fennugreek, an herb).[21] To these authors may be added Guainerio, who earlier[41] had suggested that the urine be poured on mallows or lettuce, and Beckher,[7, 10] who used mallows. Finally, *The Experienced Midwife*[3] recommends as a fertility test that the man's and woman's urine each be sprinkled on a lettuce leaf; "that which dries away first is unfruitful." ("That" presumably refers to the lettuce leaf.)

Other things than plants could be affected. Ryff[71] and Guainerio[41] recommended that a polished needle be placed overnight in a vessel of the suspected urine; next morning the needle will have reddish spots if the woman is pregnant; otherwise the needle will be black and smooth. The same test was mentioned by Jacob Rueff[69] in his volume with the reassuring title (translated) *A Nice, Cheerful Little Book of Comfort regarding the Hardships of Pregnancy and Birth of Mankind,* as well as elsewhere.[70] Rueff and Roche[64] both specify that the urine be placed in a bronze vessel. Culpeper in the next century[20] describes what is obviously the same test, but with one important difference:

> Take a handsome green Nettle, and put it into the Urin of the Woman, cover it close, and let it remain in a whole night; if the Woman be with Child, it will be full of red spots on the morrow; if not, it will be blackish.

It would be interesting to know what misunderstanding caused the substitution of *nettle* for *needle.*

In his *Dreckapotheke* of 1714 Paulini[62] attributes the same test to Wecker, and prefaces the details with a comment, here translated, which all physicians will appreciate:

> Women are always anxious to know whether they are pregnant or not—item, whether they may hope for a son or a daughter. If the doctor can give no reliable information, they have little respect for his skill.

* W. H. Ryff is not to be confused with Jacob Rueff. Ryff's *Frawen Rosengarten* was a plagiarism of Eucharius Rösslin.[67]

The test also is described in *The Experienced Midwife*[8] and, it is reported,[8] in an old Icelandic scroll. It was a practice in Hungary and among the Magyars to stick a sewing needle in a picture of the Virgin and to leave it there for nine days. If the needle were then still clean, the woman had not conceived; if rusty, she was pregnant.[65]

Another urine test for pregnancy involved spontaneous generation. A fragment in the Carlsberg papyrus[66] gives part of the procedure, which is clearly the same as for a sterility test described by Guainerio in 1500.[41] He directs both man and woman to urinate for nine days "on something full of bran"; urine from a sterile person will result in the production of worms in the bran. The papyrus says the woman should urinate on bags of sand and dates; if worms are produced she is not pregnant. Erman[33] and Iversen[66] feel that this prescription is comparable with the grain germination test, already mentioned, in the Berlin papyrus[82] and in Paulini's *Dreckapotheke*.[68] The writer does not share this opinion. Other sixteenth century authorities who described this procedure as a sterility test were Ryff[71] and the renowned Albertus Magnus.[2]

Rueff[69, 70] also outlined a related method, here translated, as a pregnancy test:

> A more certain experiment will be to bottle up the woman's urine in a glass for three days, at which time exactly it is strained through pure muslin, and if she has conceived, minute animalcules looking like lice will appear. . . .

The same procedure is described by Roche,[64] by Culpeper,[30] who suggests a rationale (". . . if you find small living creatures in it, she is most assuredly conceived with Child; for the Urin which were before part of her own substance, will be generated as well as its Mistris"), by Paulini,[68] and by the unidentified author (Culpeper?) of *The Experienced Midwife*.[8] Bartels says the test was used in Iceland.[8]

In a well-known variety of pregnancy or sterility test, the woman was dosed with a potion and her reaction was awaited. Vomiting was often a positive sign, as well it might have been; a queasy stomach is a common affliction during early pregnancy. The Egyptian papyri are again the earliest source of information. The Petrie papyri, according to Griffith,[38] date chiefly from the Middle Kingdom (about 2400 to 1580 B.C.). Prescription XXVII in this document is a fragment with crucial information missing; we only know that the woman must lie on ground wiped with the dregs of sweet beer, that an unknown procedure (not necessarily taking a potion) follows, that if she then vomits, she is pregnant, that the number of times she is sick indicates the number of children to be expected, and that if she fails to react she is sterile. Prescription VII, also fragmentary, of the

Carlsberg papyrus[46] directs that the woman drink a mixture including dates, wine, and *Sermet* (other ingredients were named in the missing section). Vomiting is a positive sign of pregnancy; flatulence, a negative indication. This test, Iversen points out, obviously is comparable with No. 193 of the Berlin papyrus, which specifies a potion of pounded watermelon and the milk of the mother of a boy.[52] (Prescription No. 194 directs that this mixture be introduced intravaginally; the same responses are to be looked for.) Brugsch's and Chabas' earlier translations give the ingredients of the potion in Prescription No. 193 as the *herbe batatu de taureau*[18] or the plant *boudodou-ka*,[13] bruised with the milk of the mother of a boy. Le Page Renouf[58] thinks that the plant belongs to the cucumber family (watermelon?); Fasbender[38] translates the hieroglyph as *watermelon seeds*.

It is most significant that, as several commentators have emphasized (see above), the Hippocratic writings contain a very similar test for fertility: ". . . if you wish to know whether a woman will conceive, give her to drink in the morning on an empty stomach some butter and milk of a woman nursing a boy; if the woman has eructations, she will conceive; if not, not."[53] Another Hippocratic test was to administer powdered red chalk and anise as a potion, followed by sleep; then pain around the navel was a sign of pregnancy. After all such tests, says Hippocrates, "the woman will drink meal, honey, and origanum in wine and oil."[53] Jacob Rueff, two thousand years later,[70] believed that vomiting after drinking the juice of a thistle was a sure sign of conception. German folk medicine advocated a comparable method. In Bavaria, apple, pear, or grape wine was allowed to stand overnight in a bowl; if the woman drank it and then vomited, she was pregnant.[53]

Hippocrates' Aphorism 41 offers another test. The woman eats no supper and drinks hydromel (water and honey) before going to sleep; she is pregnant if she is seized with abdominal pains. This test turned up again in the thirteenth century, when it was recommended by Bernard de Gordon,[36] who specified a potion of *mellicratum* (a honey preparation) and rain water and added that Avicenna had also approved the test. In 1500 Guainerio[41] described the test and added the explanation that the abdominal discomfort is due to compression of the intestines by the pregnant uterus. Other advocates were Rösslin,[66] Ryff,[71] Rueff,[70] Savonarola[73,74] ("8 oz. of rain water to 4 oz. of honey. Boil until a third part is consumed. . . ."), Bonaccivoli,[8] Roche,[64] Ambroise Paré,[56] and Culpeper.[22] Honey, curiously, was one of the several substances believed in Austria to produce *in*fertility if taken at the end of menstruation.[84]

The supposed power of a pungent odor to penetrate a woman's body via the birth canal was the basis of a large group of pregnancy and fertility tests. Such a procedure was described in Egypt as early as the Twelfth

Dynasty (about 2000 to 1800 B.C.). Iversen[48] has filled gaps in the fragmentary Prescription IV of the Carlsberg papyrus. The directions are that an onion bulb be allowed to remain all night as a pessary; if in the morning the onion odor is on the woman's breath, she will give birth. The even more fragmentary Prescription XXVIII of the Petrie papyri[50] seems to be almost identical. Iversen[48] directs attention to the closely parallel Hippocratic prescription for a fertility test: peel the outer layers from a piece of garlic and apply it as a pessary; if in the morning the woman tastes the garlic, she will conceive.[52] Or the pessary may be of wool containing a little *netopon* (oil of bitter almonds). Or a strongly flavored substance may travel in the reverse direction: if she drinks anise pounded in water and then experiences itching in the region of the navel, she will conceive.[53] Hippocrates further suggests that the lower part of the woman's body could be swathed in blankets and fumigated; "and if it appear that the scent passes through the body to the nostrils and mouth, know that of herself she is not unfruitful."[51]

Prescriptions for fumigation were turned up by Wilde[30] in ancient Irish writings, particularly in a copy made in 1496 of a book by a certain Fiongan MacTully, an hereditary physician of the province of Connaught. One procedure is "To burn the meal of Darnel (a grass) and frankincense on a red stone, and let the smoke pass through a funnel under her (a woman), and that will prepare her for conception." Wilde recognizes that the prescription probably came from a classic author. Dioscorides,[28] a physician of the first century A.D., had, as Wilde points out, recommended fumigating with a mixture of darnel (lolium), "barley flour, myrhh, saffron or frankincense as an aid to conception." Other Irish mixtures to be used in fumigation to promote (not to test for) conception were steam from the oriental narcissus (?) boiled in wine or water, or smoke from this plant burned on a red stone.

Bernard de Gordon,[36] a product of the notably unscholarly thirteenth century, reversed the Hippocratic interpretation. He described fumigation with an aromatic substance and stated that if the woman detected the odor she was *not* pregnant.

Guainerio prescribed fumigation as a fertility test in 1500.[41] He also suggested a kind of medicated pessary (more correctly, a tampon) composed of honey and *Aristolochia* or birthwort, the pungent root of which was also thought to facilitate childbirth.[41] The pessary was introduced before sleeping; the woman was judged to be fertile if on awakening she had a sweet taste in her mouth. Or, said Guainerio, she may simply sleep over a piece of garlic; if she tastes garlic when she wakens, she will be able to have a child. The Hippocratic application of pessaries and fumigation

was also advocated by other sixteenth century authorities. Rösslin[68] suggested

> the fume of some odoriferous perfume/ as laudanum/stozar/ calamyte/ lignum aloes/ muske/ ambre and suche other/ yf the odour and savour of suche thynges assende thorowe her body up unto her nose/ ye shall understande/ that sterilite commeth not of the womans parte/ yf not: then is the defecte in her.

The Rubin test seems almost to have been anticipated! Ryff[71] and Rueff[70] recommended fumigation, and Savonarola[73] mentioned fumigation and pessaries of garlic or birthwort

> 1 drachm. Let it be bruised and mixed with honey and buried [i.e., inserted] with green moss continuously until morning. Then at noontide, if while fasting her saliva grows sweet she will conceive, and similarly if it is bitter, if she does not detect its savor, she will not be pregnant.

Bonaccivoli[8] said that in fumigation the aromatic odor is transmitted via the os uteri to the nostrils. Wolff's *Harmoniae gynaeciorum,*[81] a compendium of the writings of Moschion, Priscian, and others, directed: "Insert a pessary of galbanum [a strong-smelling gum], and if the top of her head has grown warm, she can become pregnant." Rueff[70] was one of the first to call the fumigation method unreliable, but the substitute he favored was no better. Culpeper,[25] in his translation of Lazarus Riverius, condemned the latter's advocacy of fumigation. Nevertheless, it was still suggested in *The Experienced Midwife*[8] almost a century later.

In Iceland it was believed that a woman who was in doubt could let two or three drops of blood from the right side of her body fall into a stream. If they sank, she was pregnant; if they dissolved or floated, she was not.[9] Aschheim[4] says that Albertus Magnus wrote that if milk is poured on urine and if the woman is pregnant, the milk floats on top. In Lincolnshire, England, it was believed that a woman who sows parsley seed will give birth.[68]

The early Irish volume referred to previously[50] describes a quaint fertility test. If a woman could throw a pebble up onto the top stone of an ancient cromlegh, or sepulchral monument, near Dunkalk and the pebble did not fall off the smooth, convex surface, she could expect to have a child. The total number of times she would conceive would depend on the number of pebbles she could lodge on the top of the monument. The number of future children was also predicted on the basis of the number of knots or vascular enlargements in the umbilical cord.

A Nigerian procedure was one of the few in which the woman did not participate. It was the medicine man who put meal and water in a calabash,

washed his eyes with a drug, and observed in the water the answer to the pregnancy test.[78] A birth was foretold in Central Europe when a wood owl hooted above a house or a mole burrowed nearby. In a year when there were many mountain ash berries or many hazel nuts, it was expected that there would be many children.[43]

A good many methods for the recognition of pregnancy and fertility involved certain physical signs. The obvious indications of pregnancy have been well known since antiquity. Other signs, real or fancied, are both more subtle and less familiar. The Berlin papyrus suggested rubbing the woman's arms and shoulders with oil and next morning examining and palpating her arm muscles; "If the muscles of her arm thereupon contract in your hand, then you say: She has conceived."[54, 88] Other methods were to observe her blood vessels[12, 68] or feel her pulse.[82] The Petrie papyri give a vague direction for examination of the chest muscles.[85] As Dawson[24] has pointed out, both the Berlin and the Petrie papyri regard a greenish cast of the skin as a sign of pregnancy. Ryff's *Frawen Rosengarten*[71] emphasized vascular changes. Ryff and Priscian[81] believed that a warm neck and cold back were a sign of conception. The Chinese are said to have diagnosed pregnancy on the basis of amenorrhea and a particular type of pulse.[40, 97]

Yawning was a sign of beginning conception in Austria and Czechoslovakia; sneezing, of the failure of conception.[24, 45] Golden flecks on the skin were in these countries also considered sure signs of pregnancy.[21, 45] (Skin pigmentation is often altered in pregnancy[37] but the changes could scarcely be regarded as golden flecks.) Aristotle[58] described a test in which the woman's eye [lids] are rubbed with "red stone" (red chalk?); "if the substance penetrates, she is pregnant; if not, not." It is interesting that there was a Czechoslovakian superstition that if one rubs the eyelids until they are red, it is a sign of pregnancy.[45]

There has been a good deal of speculation about another "eye test." The Berlin and Carlsberg medical papyri give almost identical instructions: examine the woman's eyes while she stands in the doorway. If one eye looks like that of an Asiatic and the other like that of a Negro, she is not pregnant, but if both have the same color, then the test is positive.[52, 68] Wreszinski suggests that the woman is made to stand in the doorway so that the light will be good without the sun shining in her eyes. Lüring[54] implies in his translation that the examiner himself stands outside the house, i.e., with the light at his back. Since the eyes of the Egyptian, the Asiatic, and the Negro are dark anyway, the "difference" must have been rather subtle at best.

Classical physicians had another physical sign which does have a basis in reality; they knew that the neck swells during gestation. In suspected preg-

nancy, the diameter of the neck was measured daily. This procedure must have been common knowledge; Catullus refers to it in a poem.[60, 35, 14] The same idea appeared in the eighteenth century French medical literature: "A girl is a virgin when a thread which has been stretched from the tip of the nose to the end of the sagittal suture, at the point where it joins the lambdoidal, can then encircle her neck."[26, 42] Here, indeed, a reputation might hang by a thread! Tait[77] believes that one Natalis Guillot was the first to discover that cervical enlargement in pregnant women is due to thyroid hypertrophy; Guillot described the post-mortem findings in two cases. Thyroid enlargement is now recognized as a common occurrence in pregnancy, puberty, menstruation, and the menopause.[67, 69]

Although a few of the pregnancy and fertility tests had some basis in reality, it is evident that none of them was reliable. The failure of the tests must have been evident on the untold thousands of occasions when chance did not come to the rescue. That the tests survived, some of them for more than thirty-five centuries, is tribute to man's constant struggle to understand the phenomena of his birth.

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THE RHEUMATOID FACTOR IN SERUM AND SYNOVIAL FLUID¶

INTRODUCTION

The agglutination of sensitized sheep cells by a factor in the serum of individuals with rheumatoid arthritis has been the subject of continued investigation since its recognition by Waaler.[11] This reaction, with its modifications, furnishes the useful diagnostic tests for the illness.[1, 2, 3, 4, 9, 13]

The hemagglutination reaction has also been used in the study of the pathogenesis of rheumatoid arthritis. In this connection, efforts were made to absorb the factor from serum[6, 12] or to identify it with a serum protein fraction by virtue of differential solubility[5, 10, 13] or rate of migration.[5, 6, 7] These procedures have not been of significant help in the chemical identification of the rheumatoid factor.

Rheumatoid arthritis is characterized clinically by the prominence of changes in the synovial tissue and sometimes by the accumulation of joint fluid. Therefore, it was decided to investigate synovial fluid with the thought in mind that the joint itself might be the site of formation of the rheumatoid factor or at least a source of this material which is ultimately found circulating in the blood stream. Since the presence of mucin represents a significant difference between synovial fluid and serum, attention was also focused upon it. The viscosity, mucin clot, and mucin nitrogen to glucosamine ratio determinations reported by Ropes and Bauer[8] indicate that the hyaluronic acid of synovial fluid is depolymerized, or incompletely polymerized, in rheumatoid arthritis.

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Such alterations of hyaluronic acid in synovial fluid have not previously been related to the agglutination of sensitized sheep cells by the rheumatoid factor. This study reports the effects of *in vitro* enzymatic treatment of serum and synovial fluid in an attempt to learn if a relationship between the hemagglutination test and the mucin polysaccharide does exist.

MATERIALS

Specimens. Synovial fluid samples were obtained from the knee joints of patients at the Arthritis Clinic of the Grace-New Haven Community Hospital or from patients under the care of private physicians. Aspirations were performed, with aseptic precautions, when clinical evidence of joint effusion existed. The fluids were kept at 4° C. for 12 hours, then centrifuged. The fibrin clot, cells, and debris, if any, were discarded and the supernatant fluid stored at —10° C.

Whenever possible, blood was collected from the individual at the time of the knee tap. For the purpose of this study, however, serum and synovial fluid samples were considered contemporary if collected within one week of each other.

Enzymes. Hyaluronidase and β-glucuronidase were purchased from a commercial source.* The bovine testicular hyaluronidase had a reported assay value of 150-250 Turbidity Reducing Units per milligram. The activity of the bovine liver β-glucuronidase was given as approximately 50,000-70,000 units per gram.

Buffers. A pH 6.0 acetate buffer, 0.15 M with respect to sodium chloride, was prepared by diluting 6 ml. of 0.5 M acetic acid, 19.4 ml. of 0.5 sodium acetate and 8.77 gm. of sodium chloride to 1 liter with distilled water.

A pH 4.5 buffer was prepared from 11 ml. of 0.2 M acetic acid and 9 ml. of 0.2 M sodium acetate.

METHODS

Hemagglutination Test

A recent modification of the hemagglutination test was used.[1] It employs the settled pattern of sensitized sheep cells to indicate the endpoint of the reaction. Results are expressed in units which are the reciprocal of the test material dilution at which the last positive pattern occurred. Less than eight units is a negative test. Except for the inclusion of synovial fluids and the use of enzymes for treatment, the hemagglutination method is that previously described. To insure comparable results the contemporary samples of serum and synovial fluid were always included in the same unit of hemagglutination tests.

Enzyme Experiments

Bovine testicular hyaluronidase was dissolved in pH 6.0 buffer to give final concentration of 0.25, 0.50, 1.0, 2.5, 5.0, and 10.0 mg. per 0.3 ml. of the buffer; 0.3 ml. of the enzyme containing buffer was then added to an equal volume of serum or synovial fluid. The mixture was incubated for one hour at 37° C. Enzyme-treated synovial fluid or serum samples, along with untreated control samples, were assayed by the

* Worthington Biochemical Corporation, Freehold, New Jersey.

hemagglutination test. The final titer and the settled red cell pattern at each dilution were recorded.

A similar procedure was used with β-glucuronidase. This enzyme was dissolved, however, in pH 4.5 buffer, and the pH was readjusted with 0.1 M sodium hydroxide to approximately 6.5 after incubation and just before assay.

RESULTS

In Table 1 the hemagglutination test results of 19 contemporary serum and synovial fluid samples from 16 patients are given. It is apparent, in the series studied, that the incidence of a positive hemagglutination test in serum is greater than in synovial fluid. In the three instances in which both the serum and synovial fluid gave positive hemagglutination tests, the titer of the synovial fluid was consistently higher by one or two dilutions. From general use in this laboratory, the maximum limit of error for the test is placed at one dilution.

After preliminary studies on β-glucuronidase-treated serum and synovial fluid samples and similar samples treated with hyaluronidase, it was evident that the only treatment which significantly altered the hemagglutination test was the hyaluronidase treatment of certain synovial fluids. Therefore, the principal effort was concentrated on the effect of hyaluronidase on all available synovial fluids.

Preliminary experiments indicated that 1 mg. of hyaluronidase (150-250 T.R.U.) per 0.3 ml. of synovial fluid was a satisfactory enzyme concentration. Higher concentrations failed to elevate the titer further while lower concentrations occasionally failed to give optimal results. Control experiments showed that in the absence of the enzyme neither the pH 6.0 buffer nor the period of incubation altered the hemagglutination test. Furthermore, the buffered enzyme alone did not agglutinate sensitized sheep cells.

Table 1 also gives the synovial fluid hemagglutination test results, before and after hyaluronidase treatment, of the 16 patients with contemporary serums. In those instances (F.G., C.G.) where a positive titer was found in the synovial fluid before enzyme treatment, the action of hyaluronidase resulted either in no change or in a reduction in titer. In cases in which the serum hemagglutination test was positive but the synovial fluid test was negative, enzyme treatment of the synovial fluid resulted in a positive hemagglutination test (A.K., A.Cr., C.Fr., L.A.). The titer of the enzyme-treated synovial fluid in such cases was higher, with one exception (A.Cr.), than the titer of the corresponding serum. In addition, five hyaluronidase-treated synovial fluids had significantly positive hemagglutination tests, while the contemporary serums were negative (M.S., I.T., A.C., A.P.,

TABLE 1. COMPARISON OF THE HEMAGGLUTINATION TEST RESULTS OF CONTEMPORARY SERUMS, SYNOVIAL FLUIDS, AND HYALURONIDASE-TREATED SYNOVIAL FLUIDS

Diagnosis	*Patient*	*Hemagglutination titer**		
		Serum	*Synovial fluid*	*Synovial fluid after hyaluronidase treatment*
Group I				
Rheumatoid arthritis	F.G. 11-21-55	256	512	512
	1-16-56	512	1024	512
	11- 5-56	128	<8	512
	M.S.	<8	<8	8
	A.K.	128	<8	256
	C.G.	512	2048	256
	I.T.	<8	<8	64
	A.Cr.	256	<8	64
	C.Fr.	128	<8	256
	A.C.	<8	<8	8
	V.W. 8-30-56	<8	<8	<8
	9-15-56	<8	<8	<8
Juvenile rheumatoid arthritis	C.F.	<8	<8	<8
Group II				
Possible rheumatoid arthritis	A.P.	<8	<8	64
	H.L.	<8	<8	8
Rheumatoid arthritis or lupus erythematosus	L.A.	64	<8	512
Gonococcal arthritis history—possible rheumatoid arthritis	R.V.	<8	<8	<8
Gouty arthritis—possible rheumatoid arthritis	J.T.	<8	<8	<8
Group III				
Traumatic arthritis	S.S.	<8	<8	<8

* Expressed in units as reciprocals of serum or synovial fluid dilutions. Less than 8 units is a negative test.

H.L.). Three of these were from cases of rheumatoid arthritis and two from cases diagnosed as possible rheumatoid arthritis.

The means by which hyaluronidase produces a positive hemagglutination test was partially elucidated by examination of the actual settled sheep cell patterns. Many synovial fluids had sheep cell patterns which were atypical and could not be assigned a titer. These patterns were neither negative nor typically positive, but represented a third type of pattern which was distinct and easily recognizable. Upon addition of hyaluronidase, such fluids presented typically positive patterns and could be assigned a titer. This titer corresponded to the last dilution in which an atypical pattern appeared in the synovial fluid alone.

A few synovial fluid samples (M.S., A.C., H.L.) gave a clear-cut negative pattern in the hemagglutination test, but after treatment with hyaluronidase the hemagglutination test became typically positive at a titer of eight units.

In addition to the sixteen patients from whom both synovial fluid and serum were available, synovial fluid from fourteen other patients was studied. The hemagglutination titers of 43 synovial fluids, before and after hyaluronidase treatment, are given in Table 2.

To evaluate the specificity of the effect of hyaluronidase it was necessary to study its effect on synovial fluids of nonrheumatoid origin. Group 3 of Table 2 contains nine such patients. The post-mortem synovial fluid served as a normal control. The occurrence of a positive hemagglutination test in one case of traumatic arthritis indicates either that the effect of hyaluronidase is not entirely specific for rheumatoid arthritis or that in this case the diagnosis may change with time to that of rheumatoid arthritis.

DISCUSSION

The low incidence of a positive hemagglutination test in untreated synovial fluid and the difficulty and trauma associated with obtaining synovial fluid would seem to make it an undesirable material for the application of the routine diagnostic hemagglutination test. However, when synovial fluid is treated with hyaluronidase, prior to assay, the incidence of a positive hemagglutination test is increased, and under these circumstances synovial fluid provides at least as much information as the usual serum hemagglutination test. It may provide additional diagnostic information, but this cannot be established until more data on the specificity of the effect of hyaluronidase on the hemagglutination test are available.

Although it is unlikely that hyaluronidase acts *in vitro* to produce the rheumatoid factor, it either makes it more available for reaction with sensi-

TABLE 2. COMPARISON OF THE HEMAGGLUTINATION TEST RESULTS OF SYNOVIAL FLUIDS AND HYALURONIDASE TREATED SYNOVIAL FLUIDS

Diagnosis	*Patient*		*Hemagglutination titer*	
			Synovial fluid	*Synovial fluid after hyaluronidase treatment*
Group I				
Rheumatoid arthritis	F.G.	10-31-55	1024	512
		11-21-55	512	512
		12- 5-55	1024	1024
		1-16-56	1024	512
		3- 5-56	1024	512
		5-21-56	1024	512
		11- 5-56	<8	512
	M.S.	2-27-56	<8	8
		2-29-56	<8	<8
		3- 7-56	<8	<8
		2-26-56	<8	<8
	A.K.		<8	256
	C.G.		2048	256
	I.T.		<8	64
	A.Cr.		<8	64
	C.Fr.	4-16-56	<8	256
		5-28-56	<8	512
	A.C.	5-29-56	<8	8
		6- 6-56	<8	<8
	A.J.		<8	128
	H.H.		<8	<8
	V.W.	8-30-56	<8	<8
		9-15-56	<8	<8
Juvenile rheumatoid arthritis	F.H.		<8	16
	J.W.		<8	<8
	C.F.	9- 7-56	<8	<8
		9-12-56	<8	8
Group II				
Probable rheumatoid arthritis	D.G.		<8	<8
Possible rheumatoid arthritis	A.P.		<8	64
	N.E.		<8	16
	H.L.		<8	8
Rheumatoid arthritis or lupus erythematosus	L.A.		<8	512

Diagnosis	*Patient*	*Hemagglutination titer*	
		Synovial fluid	*Synovial fluid after hyaluronidase treatment*
Gonococcal arthritis history—possible rheumatoid arthritis	R.V.	<8	<8
Gouty arthritis possible rheumatoid arthritis	J.T.	<8	<8
Group III			
Gouty arthritis	L.C.	<8	<8
Degenative joint disease	R.P.	<8	<8
	E.M.	<8	<8
Scleroderma	G.P.	<8	<8
Traumatic arthritis	S.S.	<8	<8
	C.K.	<8	<8
	G.R.	<8	<8
	N.O.	<8	16
Post-mortem*	J.C.	<8	<8

* No history or clinical signs of joint disease.

tized sheep cells or it may depolymerize an inhibitory mucopolysaccharide. It is unclear exactly how this is accomplished, but it would appear that the effect must be ascribed to some chemical change induced by hyaluronidase. Hyaluronidase reduces the viscosity of synovial fluid by depolymerizing the polysaccharide, hyaluronic acid. The physical change in viscosity itself does not seem to be the responsible alteration, since preliminary experiments indicate that hyaluronidase can convert atypical hemagglutination patterns to typically positive ones even when a high viscosity is artificially maintained with glycerol or methocel.

SUMMARY

1. A comparison of the hemagglutination test results is made with serum and synovial fluid samples obtained from the same individual.

2. In cases in which the serum hemagglutination test was positive but the synovial fluid did not produce the usual endpoint, hyaluronidase treat-

ment of the synovial fluid resulted in a positive pattern of settled sheep cells. In addition, after hyaluronidase treatment, some synovial fluids gave positive hemagglutination tests of low titer, while the corresponding serum test did not react.

3. The effect of hyaluronidase on this hemagglutination test of synovial fluid suggests a correlation between the rheumatoid factor, or an inhibiting substance, and the mucin polysaccharide.

ACKNOWLEDGMENT

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AN UNUSUAL ECTOPIA CORDIS IN A HUMAN STILLBORN INFANT**

A stillborn female with multiple malformations had an unusual ectopia cordis unlike any that have been described insofar as the writer has been able to ascertain.

CASE HISTORY

The 39-year-old mother, whose four previous pregnancies resulted in miscarriage, was in good physical condition. Although there was moderate enlargement of her abdomen and no menses for seven months, the mother was not aware that she was pregnant until the onset of sudden, severe uterine contractions every 3 minutes of 40 seconds' duration. Upon hospital admission there was no fetal heartbeat. At the onset of labor, which lasted 11 hours and 58 minutes, the fetus was in a breech position. The membranes ruptured spontaneously, and frank bleeding occurred 55 minutes before delivery which was before full cervical dilatation. At delivery the fetus was still in a breech position with its sacrum directed toward the right acetabulum of the mother. Ectopic abdominal viscera were presented first, followed by the feet. This was the first indication of an anomalous offspring. Most of the placenta was delivered with the head to which it was attached. Episiotomy was unnecessary. The stillborn and attached portion of the placenta weighed 1 pound, 13½ ounces. The postpartum history of the mother was normal.

AUTOPSY REPORT

A midline defect in the anterior trunk wall extended from the umbilical region of the abdomen to the root of the neck where superficial extensions continued laterally to the sides of the neck (Figs. 1-2). The abdominal cavity proper was small and contained only the abdominal esophagus, an elongated gastric cardia, the portion of the colon which would normally

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** The stillbirth was brought to the attention of the author by the Department of Obstetrics and Gynecology, Yale University School of Medicine.

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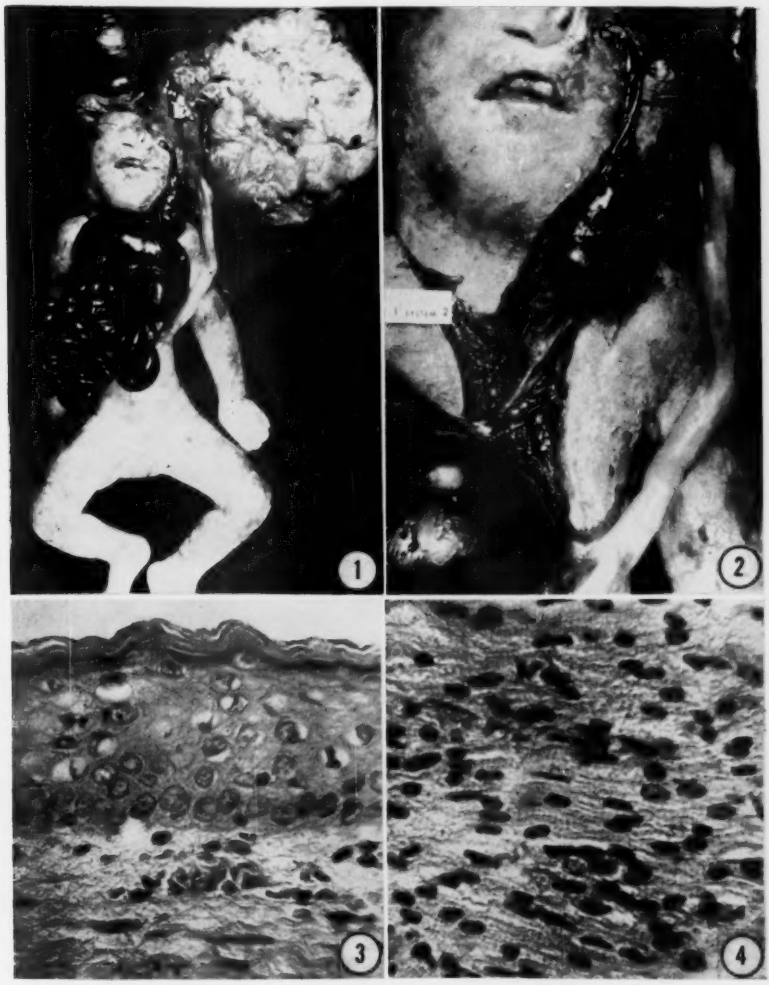

FIG. 1. Anterior view of the anomalous stillborn infant. A portion of the placenta is attached to the head. The ectopic cerebral hemisphere, inverted heart, and abdominal viscera are shown, as well as the full extent of the relatively short umbilical cord.

FIG. 2. A close-up view of the ectopia cordis. The tubular extension of the completely ectopic and inverted heart is shown passing anterior to a normal left eye to attach to the placenta. The wide defect in the thoracic wall and its lateral extension along the right side of the neck is evident. Other structures shown are the liver at the lower left, the umbilical cord, malformed left naris, and an oblique facial cleft.

FIG. 3. A section of transparent keratinized stratified squamous epithelium, lacking glands and hair follicles, which covered the defect in the thoracic wall and enveloped the ectopic heart. Underlying the stratum germinativum a portion of the thick vascular connective tissue layer which lined the heart musculature is shown. x400.

FIG. 4. A section of the tubular extension of the heart. The longitudinal arrangement of the cardiac muscle fibers is shown. x400.

have been the sigmoid, and the upper portion of the rectum. The retroperitoneal suprarenals and kidneys were in place. All the other abdominal viscera, including the pancreas and spleen within a persisting dorsal mesentery, were contained in a large, fragile, transparent sac (Fig. 1). Histologically the sac consisted of a single cell layer of amnion. Although visceral peritoneum was present on all the abdominal viscera, the parietal peritoneum did not extend beyond the intact abdominal wall into the sac. All of the intestinal tract had mesentery and because developmental rotation of the gut failed to occur no omentum was present. Within the sac the small intestine was located to the right side and the colon to the left. The liver was transposed, placing the gall-bladder on the left side of the body. The umbilical cord was abnormally short, and the two umbilical arteries were not in their usual close relationship. Also, they did not coil around the umbilical vein at any point. The abdominal wall was intact lateral to the defect and below the attachment of the umbilical cord. The pelvis and urogenital structures were normal.

The diaphragm was intact and in the area of the trunk defect it attached to a thin anterior body wall cranial to the ectopic abdominal viscera. This area of attachment was found to be, histologically, a zone of transition where the single cell-layered amnion covering the ectopic abdominal viscera became continuous with the defective thoracic wall consisting of a transparent keratinized stratified squamous epithelium which lacked glands and hair follicles. The epithelium was in contact with underlying mediastinal connective tissue and also enveloped an ectopic heart (Fig. 3). Beyond the defective wall it was continuous with the skin. The cartilageous sternum, which had only begun to ossify, was widely separated into two longitudinal parts that had failed to meet and fuse in the midline. The great vessels passed between the two halves of the sternum to attach to an inverted ectopic heart which was in turn attached to the placenta by a tubular extension of the right ventricle, measuring 2 cm. from the apex to the placenta (Fig. 2). The placenta was fused to what would normally have been the forehead. The heart was transposed for the chambers which would normally have been the right atrium and ventricle were the left and vice versa. Serial sections of the heart and its tubular extension disclosed that their surface was completely covered by the transparent keratinized stratified squamous epithelium which was intimately fused to a thick layer of connective tissue enveloping the heart musculature. Nothing resembling a serous layer of pericardium or epicardium was found. Only a 5 mm cul-de-sac of endocardium from the right ventricle continued into the tubular extension. The extension, which passed anterior to a normal left eye, was quite vascular and consisted chiefly of longitudinally-arranged cardiac muscle fibers (Fig.

4). Its ensheathing epithelium became continuous with the amnion covering the placenta to which the head was fused. The vascular ventricular extension served as a direct anastomosis between the coronary and placental vessels. A narrow mediastinum filled with areolar tissue contained the esophagus, trachea, and great vessels. The pleurae were intact, and the lungs appeared normal in their proper sites. The great vessels were elongated and showed a number of variations. The venae cavae were ventral to the arteries. The superior passed caudally to the right of the midline to join the inferior, forming a long common trunk which abruptly curved cranially to empty into the left atrium of the transposed heart. The coronary sinus also opened into the left atrium. Before the superior vena cava joined the inferior, a right and left pulmonary vein joined it. Opening into the common caval trunk were two long hepatic veins and a ductus venosus which drained the umbilical vein. Within the heart a wide patent foramen ovale was present. The membranous portion of the interventricular septum was also absent and a truncus arteriosus opened over this defect. The truncus, which passed caudally from the inverted heart, gave off two coronary arteries. No valves were present where the coronaries branched off. The truncus then made a bend dorso-cranially, giving off a short pulmonary artery containing no valves. The pulmonary artery divided into right and left branches, each of which passed to the hilus of a lung. No ductus arteriosus was found. The truncus continued cranially as the aorta to arch to the right instead of the left. A left instead of a right innominate artery was present which gave rise to the left common carotid and subclavian arteries.

Multiple anomalies of the head were also present. A narrow, shallow, oblique facial cleft ran from the medial canthus of the left eye to the upper lip which was drawn slightly upward at that point (Fig. 2). This defect represents an imperfect fusion of the frontonasal and maxillary processes during development and probably was associated with the marked elevation of the left naris. The portion of the frontal bones which normally forms the forehead, the parietals, and the interparietal portion of the occipital bone was absent. Histologically, it was found that the skin of the head continued beyond the well-formed base of the skull as a thin sac of epithelium lacking glands and hair follicles to enclose an extension of arachnoid and pia mater which contained a single, ovate cerebral hemisphere (Fig. 1). The dura mater lined the normal base of the skull but did not extend beyond it into the sac. Thus, the tentorium cerebelli was intact, but the falx cerebri and the superior and inferior sagittal venous sinuses were absent. Within the cerebral hemisphere a single tubular ventricle was present which formed a slight constriction to communicate with the antero-superior portion of a

normal third ventricle. The corpus callosum and septum pellucidum were absent. The roof of the third ventricle consisted of a layer of ependyma containing a choroid plexus which did not extend into the ventricle of the hemisphere. The rest of the diencephalon and remaining brain stem were normal. All of the cranial nerves were intact. A diaphragma sellae was present, and the sella turcica and hypophysis were normal. The arterial circle of Willis was intact; however, the cerebral arteries followed an irregular pattern on the single hemisphere. The great cerebral vein connected with a straight sinus in the tentorium which in turn joined a normal transverse sinus where the confluence of sinuses is ordinarily located.

DISCUSSION

Patten[2] speculated that ectopia cordis occurs when the heart is caught outside instead of inside the converging ventral body wall at the time of demarcation between the intra- and extra-embryonic coeloms. This would establish the body wall defect about the third week in development. The imperfect convergence of the wall apparently extended caudally to the future umbilical region of the abdomen. In the thoracic region the amnion, closing the defect in the body wall, developed into cutaneous epithelium lacking hair and gland development, whereas in the abdominal region it remained unchanged. The defective formation of the head probably also occurred early, resulting in the fusion of the head to the amnion and then in turn to the chorion in the region of the developing placenta. About the fifth week, after the heart was assumed to be caught outside the ventral body wall, the relatively large head and heart were in close proximity due to the complete flexion of the embryo. This was probably the time, if not before, when the apex of the heart fused with the placenta to which the head was already attached. Also, at this time the development of the left side of the face could have become slightly involved in the adjacent malforming processes as evidenced by the persistence of a shallow oblique facial cleft and an elevated naris. As growth changes separated the fused head and placenta from their close proximity to the thorax, the attached heart probably became completely inverted and then under additional tensional stress elongated into a tubular form in the area of the apex which was fused to the placenta.

The fusion of the head to the placenta undoubtedly caused the premature separation of the latter from the uterus during labor, resulting in frank bleeding. Fortunately the mother suffered no ill effects. If a radiogram had been made when the breech position was noted at the onset of labor or when the bleeding occurred before full cervical dilatation, the malformed skull

and sternum would have readily revealed the presence of an anomalous fetus. A similar situation existed in another study[1] where bleeding occurred during labor. A radiogram detected the presence of an anomalous fetus which was then delivered by Caesarian section.

No known factors could be definitely associated with the etiology of the anomalous stillborn, for the ancestral record and detailed information on the four previous miscarriages were lacking. The advanced reproductive age of the mother may have had little significance, as was previously reported.[1]

SUMMARY

A human stillborn female with multiple malformations had an unusual ectopia cordis. The heart was completely outside the thoracic cavity, inverted, and covered by a transparent layer of stratified squamous epithelium. A well-vascularized tubular extension of the right ventricular musculature attached to the placenta which was fused to the head, forming a direct anastomosis between the coronary and placental vessels. A possible developmental formation of the anomaly is presented.

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The Board of Editors

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REFLEX EFFECTS OF ACUTE HYPERTENSION IN THE PULMONARY VASCULAR BED OF THE DOG**

The rapid onset of severe functional disturbances of the cardio-pulmonary system so frequently seen in pulmonary embolism and primary pulmonary hypertension suggests that, in many instances, reflex activity of the nervous system may be playing a major rôle. Consequently, considerable interest has been focused in recent years upon the nature of the reflex response elicited following stimulation of the vascular bed of the lung experimentally.

Churchill and Cope[4] in 1929 were probably the first to demonstrate clearly the presence of pressoreceptors in the pulmonary vascular bed. They showed that acute pulmonary hypertension produced by rapid infusion of heparinized blood into the right pulmonary artery of the cat resulted in the simultaneous onset of systemic hypotension, bradycardia and apnea, followed by rapid shallow breathing. This response was found to be completely inhibited by bilateral vagotomy.

In 1935 Schwiegk[13] in Germany approached the problem by ligating all the blood vessels of the left lung of the dog and inserting a cannula into the peripheral end of the left pulmonary artery. He found that by raising the static pressure in the vascular bed of the lung, a reflex bradycardia and a decrease in systemic blood pressure resulted. Both of these effects required the integrity of the vagi. He also found that the reduction of systemic pressure was associated with peripheral vasodilatation and an increase in splenic volume.

Parin[11] (1947) in Moscow essentially repeated the work of Churchill and Cope. However, he made no attempt to measure the respiratory response.

Other investigators using similar methods found the response to be somewhat different than that described by the above workers. Daly and associates[5] (1937) observed that raising the perfusion pressure in the pulmonary vascular bed of dogs produced a reduction of systemic pressure, but

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a decrease or *increase* in heart rate, and occasionally an increase in respiratory rate.

Aviado and his coworkers,[2] (1951) also using perfusion techniques, reported that when the perfusion pressure of the pulmonary vascular bed of dogs was raised after first clamping the left pulmonary veins, there resulted a fall in systemic blood pressure, *no* change in pulse rate, and an increase in the respiratory rate. Perfusion at high pressures with the pul-

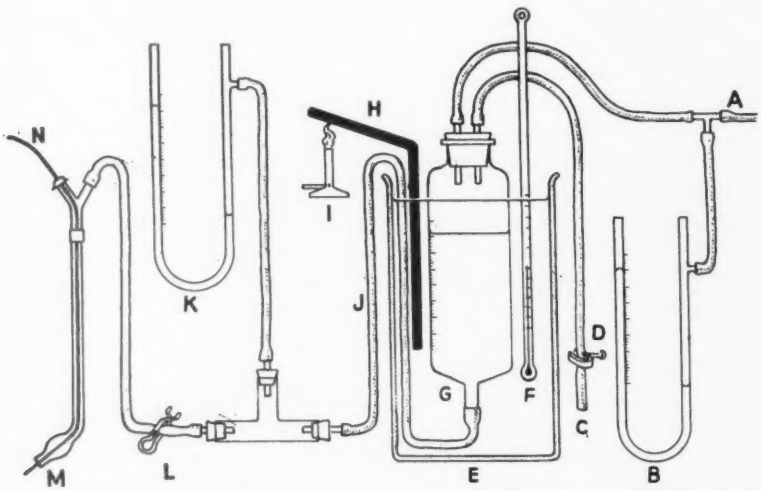

FIG. 1. Diagram of infusion apparatus. A = compressed air line. B = mercury manometer for measuring pressure in saline reservoir. C = exhaust line (air). D = screw clamp for regulating resistance in exhaust line and consequently pressure in saline reservoir. E = water bath at 37° C. F = thermometer. G = saline reservoir. H = conductor. I = heat source. J = saline infusion line. K = manometer for measuring approximate pressure in infusion line. L = pinch clamp. M = infusion cannula. N = cannula for precise measurement of pressure in left P.A.

monary veins open was found to elicit no response. These workers showed that the response was completely inhibited by ipsilateral vagotomy. They concluded that the bradycardia observed by others was due to stimulation of pressoreceptors in the region of the bifurcation of the main pulmonary artery and not to a component of the reflex elicited by increasing the pressure in the peripheral pulmonary vascular bed.

Much has been written on the subject of pressoreceptors in the pulmonary vascular bed, and it is apparent that there is a rather wide divergence of opinion regarding the nature of the reflex response. Further work on this problem, therefore, appears to be in order.

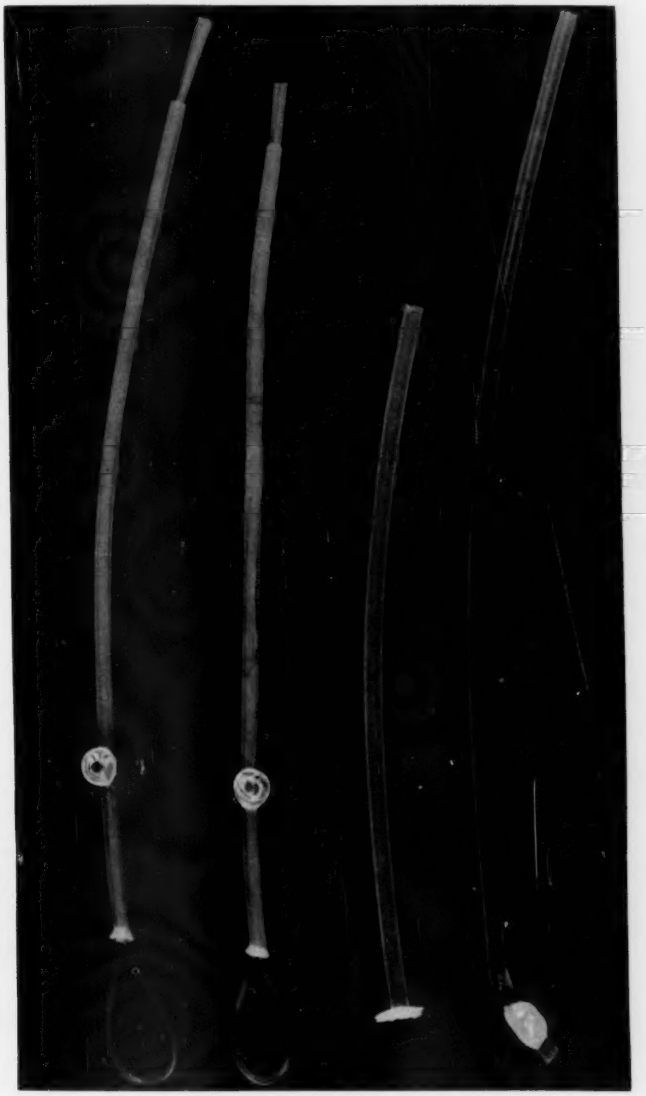

FIG. 2. Snares, guide cannula, and infusion cannula. It is to be noted that two snares were used in each preparation, one for the upper and middle lobe veins and one for the lower lobe vein of the left lung.

MATERIALS AND METHODS

The approach was to perfuse the entire left lung of the intact dog with normal saline at body temperature under variable pressures and rates of flow and to measure simultaneously (i) the perfusion pressure and rate of flow, (ii) the femoral arterial pressure, (iii) the pulse rate, (iv) the main pulmonary arterial pressure, and (v) the intrapleural pressure (as an index of respiratory rate and depth).

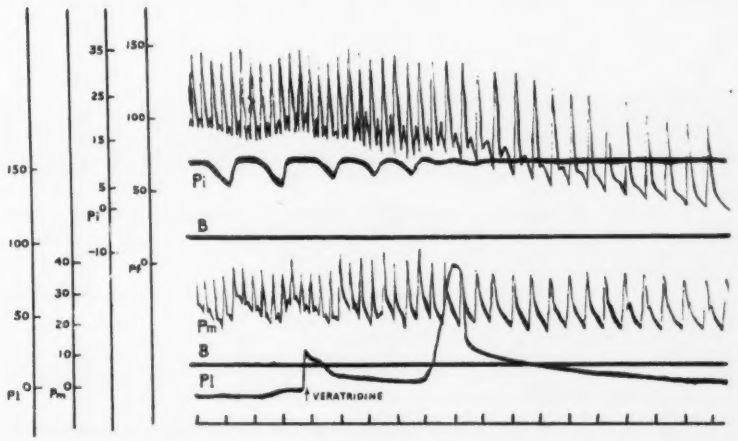

FIG. 3. Dog #444. Effect of introduction of 10 micrograms per kilogram of veratridine into the left P.A. Pf = pressure in femoral artery. Pi = intrapleural pressure. B = base lines. Pm = pressure in main P.A. Pl = pressure in left P.A. The characteristic response of this drug is seen to be a drop in systemic blood pressure, bradycardia and apnea, occurring 6 sec. after the injection. With the pulmonary veins occluded, it would appear that the response is due to the stimulation of receptors in the pulmonary vascular bed. Consequently, the material was used to determine if accidental denervation of the lung had occurred during the surgical preparation. The similarity of the response to that of acute hypertension in the same vessels is striking. The second elevation of the Pl tracing is due to the "washing in" of the drug with a few cc of saline. Time lines in seconds.

Infusion apparatus. In order that the perfusion pressure and rate of flow be accurately regulated and recorded, a special apparatus was constructed (Fig. 1). It consisted of a calibrated reservoir of infusion fluid (0.85 per cent sodium chloride solution) placed in a constant temperature bath, two mercury manometers, and the necessary tubing. Air under pressure was introduced into the top of the infusion reservoir and allowed to escape by way of an exhaust line also leading from the top of the reservoir. Hence, the pressure within the reservoir could be controlled by regulating with a screw clamp the resistance to egress of the air through the exhaust line. The pressure within the reservoir was measured with a mercury manometer. A second manometer was placed in the system between the reservoir and the infusion cannula in order to estimate the pressure in the pulmonary vascular bed at the time of the infu-

sion. This latter manometer was only for convenience, the actual pressure in the left pulmonary artery being accurately recorded by way of a cannula placed directly within the perfused vessel. Connected to the end of the infusion tubing was one limb of a glass "Y." The stem of the "Y" was attached to the infusion cannula. The other limb was sealed by a rubber diaphragm. A #15 needle was used to perforate the diaphragm, and through this was passed a small cannula to extend through and 1 cm beyond the end of the infusion cannula into the left pulmonary artery. When the needle was removed from the diaphragm, a water-tight seal was left. This cannula so placed was used to measure the induced pressure in the left pulmonary vascular bed.

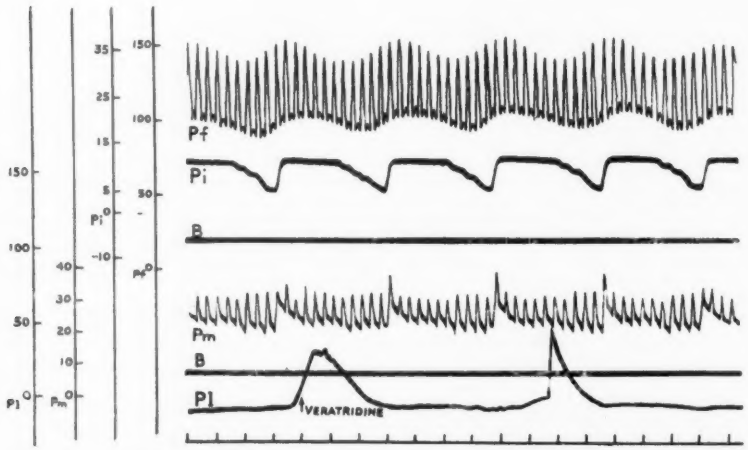

FIG. 4. Dog #444. Introduction of 10 micrograms per kilogram veratridine into left P.A. after ipsilateral cervical vagosympathectomy. Compare with Fig. 3. Pf = pressure in femoral artery. Pi = intrapleural pressure. B = base lines. Pm = pressure in main P.A. Pl = pressure in left P.A. It is seen that the effects of veratridine as shown in Fig. 3 are abolished by denervation. Time lines in seconds.

Calculation of perfusion pressure. In those instances where the perfusion pressure could not be recorded for technical reasons, the pressure was determined by the formula: $R = \frac{P_1 - P_2}{F}$ or expressed differently: $P_2 = P_1 - RF$ where P_2 is the pressure at the orifice of the infusion cannula; P_1 is the pressure in the saline reservoir; F is the measured rate of flow of the saline through the infusion apparatus; and R is the resistance to flow through the infusion apparatus for that rate of flow that occurs during the infusion. This rate was established by measuring the flow through the apparatus at different reservoir pressures when the pressure at the orifice of the cannula was zero (atmospheric). A graph expressing the resistance to flow through the apparatus in relation to the rate of flow was constructed utilizing the first formula above when the pressure at the orifice of the cannula was zero. From this graph could be read the R for that rate of flow that occurred during an infusion. This could then

be used as "R" in the second formula in order to calculate the P_s, or pressure, at the orifice of the infusion cannula.

Inspection of the data shows that where comparison of the recorded and calculated perfusion pressures is possible, the figures were in agreement generally, differing from 0 to 9 mm Hg, with one exception in which the difference was 23 mm Hg.

Cannulae and snares. In order that the experiment might be performed on animals with thorax closed, it was necessary to construct various cannulae (Fig. 2) accessible from the skin. Consequently, into each animal was surgically placed (i) an infusion

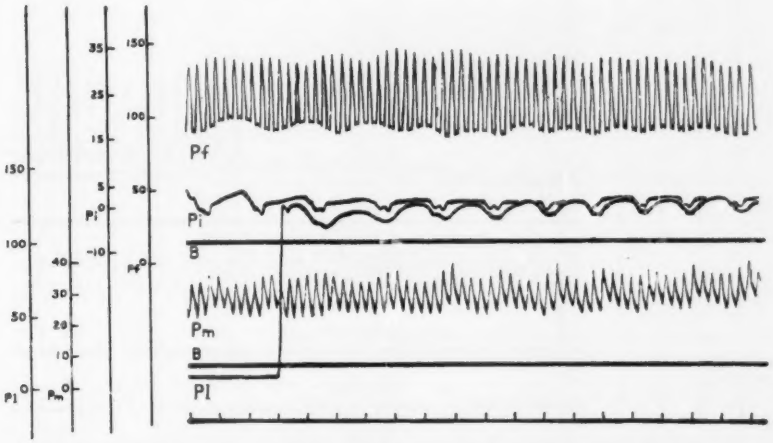

FIG. 5. Dog #444. Events following infusion of 100 cc saline with the pulmonary veins open. Pf = pressure in the femoral artery. Pi = intrapleural pressure. B = base lines. Pm = pressure in main pulmonary artery. Pl = pressure in the left pulmonary artery (infusion pressure). Minimal fluctuation of systemic blood pressure and no change in heart rate are observed after rapidly raising the pressure in the left P.A. from 0 to 116 mm Hg. There is no change in respiratory rate, the diminution of depth probably being due to the volume of fluid introduced. The main P.A. pressure is seen to rise only slightly. Time lines in seconds.

cannula into the left pulmonary artery, (ii) a guide cannula sutured to the main pulmonary artery through which a 19-gauge lumbar puncture needle could be passed for the purpose of measuring the main pulmonary arterial pressure,[8] and (iii) two specially designed snares for the pulmonary veins, by means of which the venous return of the left lung could be occluded or opened at will. With these aids, the necessary manipulations could be performed from the exterior of the animal.

Veratridine. Veratridine, a member of the veratrum alkaloid group, known to stimulate certain receptors in the cardiovascular system,[10, 9, 6, 1, 7, 8] was used to ascertain whether denervation of the pulmonary vascular bed had occurred accidentally during surgical manipulations in the region of the hilar plexus of nerves. This compound was shown by Dawes, Mott, and Widdicombe[6] and others to produce the sudden onset of apnea, bradycardia, and hypotension when injected into the vascular system, this being

a reflex response dependent upon the integrity of the vagi. Aviado[1] showed that injection of the substance into the pulmonary artery produced immediate apnea, and that the bradycardia and hypotension did not occur until approximately six seconds after the injection. He concluded that the apnea was due to the stimulation of receptors present in the pulmonary vascular bed. This latter finding was used as a method of demonstrating the probable presence of intact pathways over which a reflex response could be mediated. Any failure of response would not, therefore, be on the basis of accidental denervation during the surgical procedure. For this purpose, veratridine in

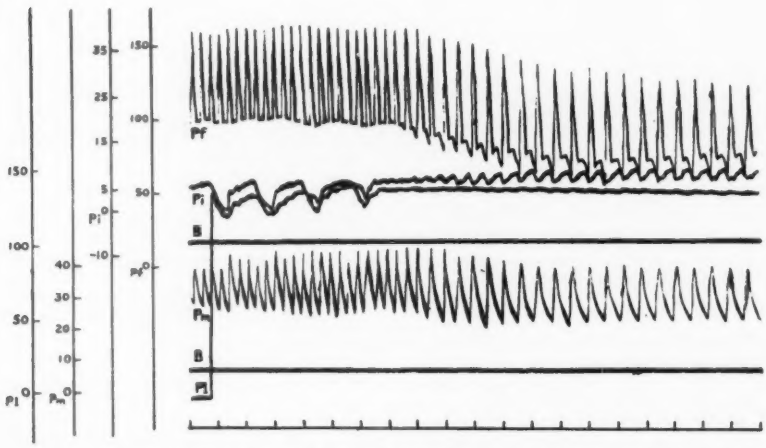

FIG. 6. Dog #444. Results of infusion of 100 cc saline with left pulmonary veins occluded. Pf = pressure in femoral artery. Pi = intrapleural pressure. B = base lines. Pm = pressure in main pulmonary artery. Pl = infusion pressure in left P.A. The pressure in the left P.A. is raised from 0 to 142 mm Hg. The systemic blood pressure is seen to drop from a mean of 126 mm Hg to 88 mm Hg, the heart rate to drop from 180/min. to 90/min., and the respirations to cease. These responses have their onset simultaneously 6 sec. after starting the infusion. It is observed that the main P.A. pressure drops 2 to 3 mm Hg. Time lines in seconds.

doses of 10 micrograms per Kg was injected into the left pulmonary artery after closing the snares and occluding the venous return from the lung (Figs. 3 and 4).

Preparation of animals. The dogs were divided into two groups, chronic and acute. In the former, the animals were prepared surgically under the usual aseptic conditions ten to fourteen days prior to the experiment. Fourteen dogs were prepared in this group, of which four could not be used for various technical reasons. Eight were prepared for acute experiments. In this latter group, one could not be used because of death immediately following the surgical preparation. It is important to note that, in the acute as well as the chronic group, the chest was closed and the animal allowed to breathe room air entirely at his own volition. It would appear, therefore, that the two groups are comparable in this respect in terms of responses obtained. The effects of pulmonary hypertension induced by rapid saline infusion on the animals prepared in this manner were recorded by means of a Hathaway multichannel electronic recorder.

EXPERIMENTAL PROCEDURE

The animals were anesthetized very lightly with Na thiopental intravenously titrated in such a way that they maintained an active eyelid reflex. An incision was made in the skin overlying the ends of the cannulae and the latter were exposed. A #19 lumbar puncture needle was passed through the guide cannula into the main pulmonary artery. The infusion apparatus was attached to the left pulmonary artery cannula. The left femoral vessels were cannulated, the femoral artery being used for recording systemic pressure changes and the femoral vein for infusion of the anesthetic. A small polyethylene cannula was inserted through the right chest wall through a #15 needle for recording intrapleural pressure changes. The left pulmonary artery infusion pressure was recorded by passing a polyethylene cannula with internal diameter of .034 inches into the left pulmonary artery through the lumen of the infusion cannula. The cannulae were then attached to transducers of the electronic recorder and the various pressures recorded. It is to be observed that the animal was breathing air spontaneously and was otherwise an essentially intact, normal dog, with the exception of having only one lung (the right) available for respiratory exchange. The animals were not heparinized systemically, but heparin was used in the various cannulae to keep them free of clot.

The experiment was performed in the following way:

1. Base lines were obtained
2. The Hathaway machine was standardized
3. 100 cc of normal saline at 37° C. were introduced into the left pulmonary artery at a pressure of 200 mm Hg in the saline reservoir
4. The pulmonary veins of the left lung were occluded by pulling up the snares
5. 100 cc saline at 200 mm Hg, as above
6. 100 cc saline at 160 mm Hg, as above
7. 100 cc saline at 100 mm Hg, as above
8. Right vagosympathetic nerve severed in the cervical region
9. 100 cc saline at 200 mm Hg, as above
10. 10 micrograms per kilogram body weight of veratridine introduced into the left pulmonary artery.
11. Left vagosympathetic nerve severed in the cervical region
12. 100 cc saline at 200 mm Hg introduced, as above
13. Veratridine, 10 micrograms per Kg introduced, as above
14. Machine recalibrated
15. Animal sacrificed.

It should be mentioned that after each infusion the pulmonary vein snares were opened in order to prevent the development of pulmonary edema from loading the pulmonary vascular bed with several hundred cubic centimeters of saline. Ample time was allowed following reclosure for equilibrium to occur.

RESULTS AND DISCUSSION

Twenty-two animals were prepared as described. Of these, five died of various causes prior to the recording. In the first six of the recorded series, no response was obtained because of the very small rate of perfusion obtained (less than 0.5 cc per second) through the small perfusion can-

nulae #205 (internal diameter of 0.062 inches) utilized in the first eight dogs of the series. Larger cannulae with an internal diameter of 0.115 inches were used in the subsequent preparations. In two of the animals prepared with the larger cannulae, the cannulae were found at post mortem to have slipped out of position, one into the left lower lobe and one into the lingular lobe. No response was observed when these animals were perfused.

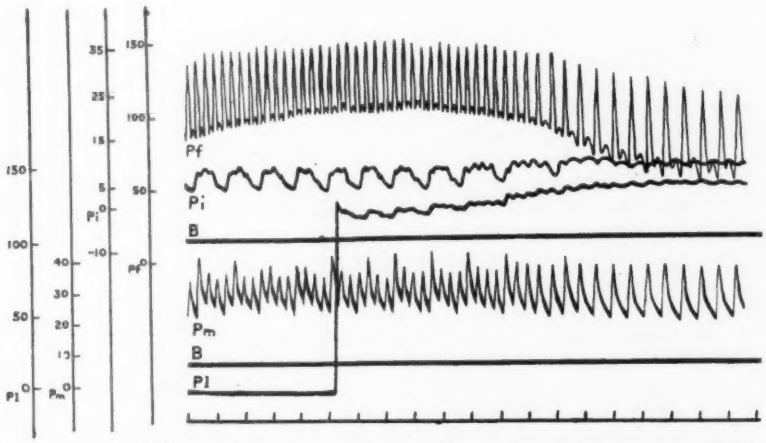

FIG. 7. Dog #444. Results of introduction of 100 cc saline. Pulmonary veins (left) occluded. (Fourth infusion.) Pf = pressure in femoral artery. Pi = intrapleural pressure. B = base lines. Pm = pressure in main P.A. Pl = pressure in left P.A. (infusion). This represents a repetition of the infusion shown in Fig. 6. A drop of systemic pressure of 38 mm Hg, bradycardia (180 to 90/min.), and apnea are observed. The main P.A. pressure is seen to drop slightly. The left P.A. pressure in this infusion is also 142 mm Hg. The onset of the response is 6 sec. after the start of the infusion. Time lines in seconds.

Twenty-nine observations were recorded on the nine remaining dogs in the manner described above, which demonstrated responses to acute pulmonary hypertension (Figs. 6, 7, and 8). Fourteen responses were observed in the four chronic dogs and fifteen responses in the five acute experiments. In the first two dogs of the chronic group, the smaller cannulae were used and the infusion pressure was not recorded. However, when adequate flow and, consequently, adequate pressure were obtained by using very high pressures in the infusion reservoir, a striking response occurred. In all of the chronic animals with the infusion cannulae in the left pulmonary artery for ten to fourteen days, clotting occurred in the region of the orifice of the cannula to a variable extent. This was cleared as much as possible by aspiration, utilizing a cannula passed through the infusion

TABLE 1. RESPONSE TO ELEVATION OF PRESSURE IN THE LEFT PULMONARY ARTERY

Dog #	*Weight (Kg)*	*Time*	*Flow (cc/sec.)*	*Recorded LPAp in mm Hg*	*Calculate LPAp in mm Hg*	*Respiratory Response/min.**	*Decrease in heart rate in beats/min.*	*Decrease in blood press. in mm Hg*	*Saline reservoir pressure in mm Hg*	
					Chronic Group					
395	18									
397	21									
398	13				(In this group of animals, a flow of not over 0.5 cc per second of perfusant					
419	15				was given. No response occurred because of inadequate rate infusion.)					
427	19									
1–0	14									
430	12	10:00	3.3	...	...	70-60-70	10	16	...	
432	12	10:00	3.6	...	...	50-30-50	5	21	...	
"	12	10:15	3.8	...	...	40-0-80	18	12	...	
444	13	12:44	3.3	142	135	40-0-45	90	37	200	
"	13	12:50	3.8	142	119	50-0-50	90	38	200	
"	13	1:10	2.7	107	111	55-35-55	90	22	160	
"	13	1:15	0.9	80	80	60-50-60	30	12	100	
"	13	1:27	3.8	123	119	38-50-45	60	30	300	(Rt. vagus cut)
445	17	12:55	4.0	114	112	20-0-20	20	7	200	
"	17	1:05	3.0	138	143	25-0-25	36	13	200	
"	17	1:13	3.6	122	125	50-0-40	30	0	200	
"	17	1:20	3.9	134	136	10-0-10	15	13	220	(Rt. vagus cut)
"	17	1:31	3.6	130	125	10-0-10	16	7	200	(Rt. vagus cut)
"	17	1:37	2.0	82	74	10-0-10	24	20	160	(Rt. vagus cut)

* As the respiratory response is expressed here, the first figure indicates the rate per minute prior to the infusion, the second figure is the rate during the infusion (0 indicates a period of apnea), and the third figure the rate following the infusion when the animal has returned to a relatively steady state.

LPAp indicates the perfusion pressure in the left pulmonary artery, as recorded and/or calculated.

cannula. However, there was always some clot left in the vessel which narrowed its lumen at a point slightly distal to the orifice of the infusion cannula. This obstruction resulted in an unusually high perfusion pressure just proximal to the clot and suggests that the remainder of the vascular bed was being perfused at a much lower pressure relative to this proximal segment. This interpretation is borne out when one compares the pressure required to produce comparable responses in the chronic and the acute groups. For example, in dog ♯444 of the chronic group (Table 1), a recorded pressure of approximately 135 mm Hg produced a drop of 37 mm

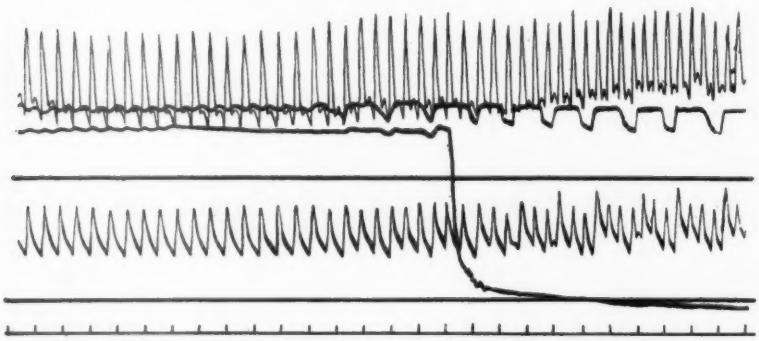

FIG. 8. Continuation of Fig. 7. About 0.4 sec. of recording between the two figures not included. It is seen that there is 'vagal escape' in that the systemic pressure, the heart rate and the respirations begin to return to the pre-infusion state while the pressure in the left P.A. is still elevated. Time line in seconds.

Hg in systemic pressure. Whereas in dog ♯7-0 of the acute group (Table 2), the same degree of hypotension resulted from a pressure of only 42 mm Hg in the left pulmonary artery. Also, evidence of increased resistance to flow is suggested in comparing dog ♯444 of the chronic group (Table 1) in which a left pulmonary artery pressure of 80 mm Hg produced a flow of only 0.9 cc per second, while a comparable pressure of 86 mm Hg in dog ♯5-0 of the acute group (Table 2) resulted in a flow of 3.8 cc per second.

It is to be noted that in none of the animals of either group was a response observed when perfusion of the lung was carried out with the venous return unobstructed (Fig. 5).

The results showed an average of 1 mm Hg drop in systemic pressure for each 2.4 mm Hg elevation of perfusion pressure in the left pulmonary artery with a range of 1 to 1.1 to 1 to 3.1. This compares closely with the findings of Churchill and Cope who found a 1 mm Hg drop in systemic pressure for each 2.3 mm Hg rise in perfusion pressure in the cat.

Bradycardia was found almost consistently to be a component of this

TABLE 2. RESPONSE TO ELEVATION OF PRESSURE IN THE LEFT PULMONARY ARTERY

Dog #	*Weight (Kg)*	*Time*	*Flow (cc/sec.)*	*Recorded LPAp in mm Hg*	*Calculated LPAp in mm Hg*	*Respiratory response/ min.**	*Decrease in heart rate in beats/min.*	*Decrease in blood press. in mm Hg*	*Saline reservoir pressure in mm Hg*	
					Acute Group					
4–0	12	10:30	5.2	...	45	10-0-10	60	38	200	
4–0	12	10:45	1.9	...	56	10-0-10	30	15	100	
5–0	22	1:18	3.8	...	86	55-96-60	0	8	200	
6–0	20	7:37	5.5	...	36	50-80-55	65	0	200	
"	20	7:42	5.5	...	36	50-75-50	45	0	200	
7–0	12	1:43	5.3	...	42	50-95-55	68	38	200	
"	12	1:50	3.8	...	58	50-65-50	30	23	160	
"	12	2:00	5.3	...	42	40-60-45	30	14	200	
"	12	2:15	5.3	...	42	12-40-20	0	13	200	
8–0	12	12:05	5.0	59	50	38-20-35	45	21	200	
"	12	12:07	4.3	46	45	30-12-30	25	3	160	
"	12	12:10	5.0	46	50	38-15-25	25	16	200	
"	12	12:17	5.0	51	50	18-8-18	20	9	200	(Rt. vagus cut)
"	12	12:36	4.5	37	39	14-12-14	8	10 (incr.)	160	

* As the respiratory response is expressed here, the first figure indicates the rate per minute prior to the infusion, the second figure is the rate during the infusion (0 indicates a period of apnea), and the third figure the rate following the infusion when the animal has returned to a relatively steady state.

LPAp indicates the perfusion pressure in the left pulmonary artery, as recorded and/or calculated.

reflex (27 out of 29 observations) in contrast with the observations of Daly *et al.*, who found a decrease *or increase* in heart rate, and with Aviado, who found *no* change in the heart rate. It was the suggestion of Aviado that the other workers who had found bradycardia as part of the response may have been stimulating receptors in the region of the bifurcation of the pulmonary artery. The pressure recordings in the main pulmonary artery

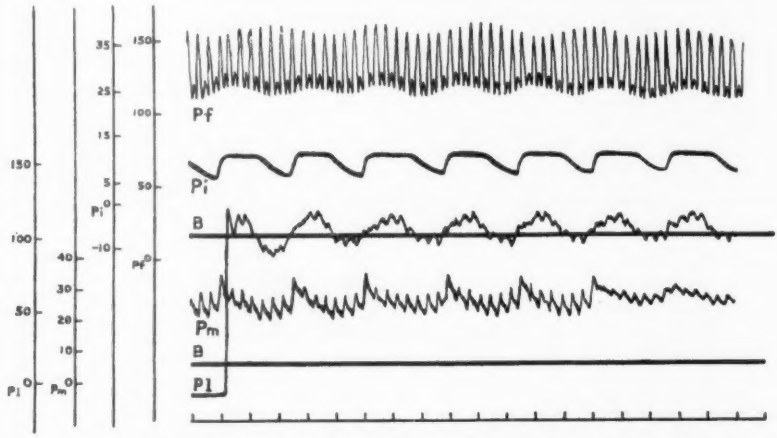

FIG. 9. Dog #444. Result of infusion of 100 cc saline after ipsilateral cervical vago-sympathectomy. Pulmonary veins occluded on left. Pf = pressure in femoral artery. Pi = intrapleural pressure. B = base lines. Pm = pressure in main P.A. Pl = pressure in left P.A. It is seen that raising the pressure in the left P.A. to 120 mm Hg produces no effect on the systemic blood pressure, heart rate or respiration following denervation. Time lines in seconds.

showed a rise of not more than 3 mm Hg, and, more commonly, the pressure in this vessel fell during the infusion. Therefore, in agreement with Churchill and Cope, it is concluded that bradycardia is a part of the response to hypertension in the pulmonary vascular bed distal to the bifurcation of the main pulmonary artery.

The effects of the perfusion on the respiration of the animals were not as consistent as the other findings. Churchill and Cope found that a period of apnea followed by rapid, shallow breathing occurred in the cat. Aviado found that the response was characterized by an increase in the respiratory rate without a preceding period of apnea. In the present study it was found that, in six of the nine dogs, the response was one of depression, apnea, or bradypnea, followed in a few seconds after the infusion had been terminated by a resumption of the original rate, or nearly so. The period of apnea, when it occurred, lasted from 10 to 20 seconds. In three of the animals,

however, the response was that of rapid, shallow breathing during the infusion, followed by a resumption of the original rate. The reason for this difference in response is not readily apparent from the data.

It was observed that all the components of the reflex response occurred simultaneously six to seven seconds after starting the infusion. Severing the contralateral vagosympathetic nerve in the cervical region did not appear to inhibit the reflex. However, severing the ipsilateral nerve abolished the response entirely (Fig. 9).

In accord with the findings of Daly and co-workers and Aviado and associates, a response to the high perfusion pressure did not occur when the pulmonary vein snares were left open and the venous return of the lung was unimpeded (Fig. 5).

An additional observation of interest is that of the phenomenon of "vagal escape." In Figure 8 it is seen that the heart rate and blood pressure have begun to return to pre-perfusion values and that respiratory activity has returned while the pressure in the left pulmonary vascular bed is still elevated to the same level as that at the beginning of the perfusion.

Of 24 instances where such an observation could be made the phenomenon was observed seven times. This includes at least one such observation in five out of six animals. Twenty-two seconds or more (22 to 33) of perfusion was required before "escape" occurred with one exception in which the phenomenon appeared after only 16 seconds of perfusion. Why this phenomenon was not observed during the remaining 17 perfusions may be related to numerous ill-defined factors. In nine of them the period of perfusion was 22 seconds or less. This may represent insufficient time for the phenomenon to occur in view of the fact that in two of the seven instances where "escape" did occur, 31 and 33 seconds respectively of perfusion were required. In only four out of the 24 perfusions was the phenomenon not observed when the perfusion was prolonged for more than 31 seconds. The reason for the failure of "escape" to occur in these four instances is not readily apparent.

This observation may help to explain the brief nature of the episode of shock, bradycardia, and respiratory arrest frequently seen in cases of primary pulmonary hypertension. It may also help to explain why patients with other forms of chronic pulmonary hypertension do not manifest continuous bradycardia, systemic hypotension, and respiratory difficulties.

SUMMARY AND CONCLUSIONS

This study demonstrates that the response of the intact dog to acute hypertension in the peripheral pulmonary vascular bed consists of three components:

1. A drop in systemic pressure
2. Bradycardia
3. A change in the respiratory pattern (depression or stimulation).

All of the components have their onset simultaneously about 6 to 7 seconds after beginning the infusion. None of the components is observed to occur following ipsilateral vagotomy.

Bradycardia occurs as a component of the reflex in the absence of increased pressure in the main pulmonary artery.

The phenomenon of "vagal escape" was found to be characteristic of this reflex.

Veratridine was used to demonstrate the presence of intact nerve pathways.

The fact that the response occurs only when the pulmonary venous drainage is obstructed would suggest that the majority of the receptors are located in the distal pulmonary vascular bed.

This study corroborates in the dog the original findings in the cat of Churchill and Cope regarding the response of the cardiovascular system to acute pulmonary hypertension. The respiratory response, however, was found to be less consistent in character and somewhat different than that described by these investigators.

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STUDIES ON THE PATHOGENESIS OF VASCULAR DISEASE

The Effect of Intravenously Injected Human Plasma and of Lipid-Rich Human Plasma Globulins on Inflammatory Lesions of the Coronary Arteries of Dogs**

Investigations of Gofman and his associates[4] have directed attention to the plasma lipoproteins in persons with clinical manifestations of arteriosclerosis. Recently, lipoproteins with flotational characteristics similar to those in serum have been extracted from diseased human aortas.[5] Some direct evidence for the participation of these substances in the genesis of experimental arterial changes in animals has also been reported: Bragdon[1, 2, 3] injected rats intravenously with material derived from plasma lipoproteins of hypercholesterolemic rabbits and obtained scattered fatty arterial lesions. The experiment in rats was repeated, substituting human lipoprotein fractions, with a similar result. From this laboratory preliminary report has been made of experiments indicating that lipids localize selectively in areas of concomitant arterial injury in dogs following the intravenous injection of human plasma or of lipoprotein fractions.[15, 16, 17] The report which follows describes these studies in further detail.

The general plan of the investigation has been to observe the effect of intravenously injected human plasma or of lipid-rich human plasma globulins on the basic morphological sequences of lesions of dogs' coronary arteries injured by the administration of allylamine.

MATERIALS AND METHODS

Adult mongrel dogs maintained on a low-fat, standard laboratory diet were utilized. The technique for the production of lesions of the coronary arteries of dogs by allylamine has been described previously.[12] In brief, 15-20 mg./kg. of neutralized allylamine in a 1 per cent aqueous solution were injected into dogs intravenously on each of the first three days of the experiment, or on days 1, 3, and 4, or on 1, 2, and 4,

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depending on the tolerance of the animal. With this regimen, 80-90 per cent of the animals exhibited multiple lesions of their coronary arteries when examined from three days to two weeks after the beginning of the experiments. In any one animal the lesions varied in intensity, ranging from small areas of medial edema and necrosis to necrotizing panarteritis with intimal proliferation and partial or complete obliteration of the involved segment. Control allylamine lesions contained at most traces of stainable fat and progressed to scar.

Clear human "plasma" for injection was prepared by centrifuging recently outdated, but nonhemolyzed ACD blood (whole blood approximately 440 ml., Anticoagulant Acid Citrate Dextrose Solution B., U.S.P. 110 ml.) at low speed and by subsequently separating the plasma-preservative mixture. The latter was now centrifuged at 20,000 r.p.m. for one hour to remove large fatty particles (chylomicrons) and any other insoluble material. In a group of eight dogs, following the allylamine injections, the clear plasma-preservative mixture was given over periods of from 30 minutes to seven days in total amounts varying from 300 to 5,125 ml. (See Table 8). Such acute augmentation of blood volume may in itself lead to damage of dogs' coronary arteries, as has been demonstrated.[12] However, the combined vascular-damaging effects of allylamine and of this procedure were not appreciably different from those of allylamine alone.

Lactescent human plasma was obtained by centrifuging chylomicron-rich bank blood at low speed and by separating the plasma with precautions for sterility. Lactescent plasma was administered to a series of five allylamine-injected animals in varying large amounts as set forth in Table 9.

The starting material for the preparation of lipid-rich protein fractions of human plasma was also sterile ACD bank plasma from recently outdated, nonhemolyzed bank blood. After centrifugation at 20,000 r.p.m. for an hour to remove chylomicron-like particles, the plasma was treated with an aqueous solution of polyethylene glycols (carbowaxes*) which effected, at room temperature and at a pH of 6.7-6.9, the precipitation of an undenatured lipid-rich protein complex. The precipitant polyethylene glycol solution was prepared as follows: To nine parts of melted carbowax 4,000, one part of melted carbowax 1,500 was added. An equal volume of sterile distilled water was added to the melted glycols, a 50 per cent aqueous solution (pH 5.2) of the mixture resulting. This preparation was used in the precipitations to be described. (The proportions of carbowax apparently may be varied as certain other mixtures have also proven effective. However, a survey of optimal polyethylene glycol preparations for the precipitation of the protein fractions of plasma was beyond the scope of the present investigation.)

For precipitation of the lipid-rich plasma protein fraction utilized in these experiments, 25 ml. of the 50 per cent aqueous carbowax solution were slowly added with stirring to each 100 ml. of plasma. After complete precipitation, the yellow-orange precipitate was separated by centrifugation. From four samples of ACD human plasma, the average recoveries of lipids and of proteins in the fraction were as shown in Table 1.

The crude, lipid-rich plasma protein precipitate was readily soluble in physiological saline and upon the addition of water. This precipitate, dissolved in 0.9 per cent saline,

* Carbowaxes are products of the Union Carbide and Carbon Corporation, New York, New York.

was used in the first few animals, but thereafter further purification was carried out. No differences in the physiological or pathological effects of the crude or more purified materials were noted. In preparation for the majority of the experiments, the first carbowax precipitate was dissolved in a volume of distilled water equal to the original plasma volume and was then allowed to stand overnight at 4° C. A lipid-poor, gray-white precipitate separated. This was removed by centrifugation, and the clear, light-yellow supernatant was reprecipitated with 50 per cent aqueous carbowax as before. The resultant precipitate was homogenous in texture and light-yellow in color. Its solubility was such that five- to sixfold concentrates in saline were possible. As a

TABLE 1. LIPIDS AND PROTEIN REMOVED FROM ACD PLASMA BY PRECIPITATION WITH POLYETHYLENE GLYCOLS (AVERAGE OF 4 SAMPLES)

	Per cent of total
Total cholesterol	82.8
Free cholesterol	100.0
Fatty acids	58.1
Lipid phosphorus	51.1
Protein	30.7

Actual Values Obtained in a Sample Analysis

Material	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*	*Total protein gm.%*
Control plasma	174.3	31.5	9.7	7.1	5.2
Carbowax fraction	138.7	36.8	6.3	4.1	1.6
Duplicate fraction	138.7	33.8	6.3	3.8	1.2
Triplicate fraction	132.5	33.8	6.4	3.7	.95

usual procedure for injection, the precipitate from 1,800 ml. of blood-bank plasma was redissolved in 300 ml. of 0.9 per cent saline. On standing, a slight lipid-poor precipitate separated. After removal of this material, the clear, sterile concentrated protein solution (pH 6.5-6.7) was utilized for injection. The pertinent components of a typical concentrate are given in Table 2.

The majority of the plasma protein solutions utilized contained residual amounts of the precipitant glycols. However, the injection of this fraction into dogs in concentrated form did not lead to the known renal or hepatic lesions of polyethylene glycol poisoning. In control experiments it was found that the amount of injected pure glycol associated with the appearance of such lesions in dogs greatly exceeded that present in the lipid-rich protein fractions.

In any case, experience was gained with a method for the removal of residual polyethylene glycols from the lipid-rich plasma globulin fractions. The injection of this carbowax-free material resulted in the same vascular lesions as the less purified globulin fractions (See Table 10, Dog No. 1982.). Glycols were removed from the

fractions by the following procedure: Initial precipitates from blood-bank plasma were dissolved in distilled water and were allowed to stand overnight at 4° C. A gray-white, lipid-poor precipitate was separated by low-speed centrifugation. The supernatant was then treated with the glycol mixture. The resultant precipitate was washed repeatedly with distilled water in which, as a mixture of globulins, it was only sparingly soluble. Three to five washings sufficed to remove carbowaxes from the fraction. Completeness of removal was determined by the sensitive colorimetric method of Shaffer and Critchfield.[11] Losses of lipid-containing proteins in the supernatants, particularly in the first washing, were considerable. This appeared to be a matter of ionic strength, since the globulin could be partially recovered by dilution or by dialysis of the supernatant.

Table 2. Lipid and Protein Content of a Plasma Protein Concentrate Used for Injection

Total cholesterol	725.0 mg.%
Free cholesterol	224.9 mg.%
Fatty acids	40.0 mEq./l.
Lipid phosphorus	23.4 mg.%
Total protein (globulin)	7.0 gm.%

Table 3. Recovery of Lipids and of Protein in Carbowax-free Globulin Fraction of ACD Human Plasma (average of four samples)

	Per cent of total
Total cholesterol	58.6
Free cholesterol	66.7
Fatty acids	49.3
Lipid phosphorus	55.2
Protein (globulin)	7.5

Without reworking of the supernatants, recoveries of plasma lipids and of proteins in the purified material from bank plasma were as shown in Table 3.

Not only was the residual carbowax removed, but there was also elimination of much lipid-poor protein from the fraction. Large variation in the amounts of lipids recovered occurred among samples. This was believed to be associated at least in part with differences in the age and in the composition of the original plasma-preservative mixtures.

The procedure described for the preparation of a carbowax-free protein fraction of blood-bank plasma was also effective with fresh human serum or with fresh dog serum. However, with these materials the precipitations often occurred more slowly, and high speed centrifugation was sometimes necessary to effect the separations. Of considerable interest was the observation that the glycol-precipitable fraction of fresh

dog serum contained, in contrast to that from human serum, only a small fraction of the total serum lipids.

The washed, carbowax-free, lipid-rich protein fraction from human plasma was soft, sticky, and a homogeneous bright yellow in color. In saline, at pH 6.5-6.7 and on standing, a further fine nonlipid-containing protein precipitate separated. This did not occur in phosphate buffer at pH 7.4. At room temperature, ether did not extract appreciable quantities of lipid from saline solutions of the fraction. However, when precipitated

TABLE 4. LIPID AND PROTEIN CONTENT OF A CARBOWAX-FREE GLOBULIN CONCENTRATE FROM HUMAN PLASMA

Total cholesterol	610.0 mg.%
Free cholesterol	180.0 mg.%
Fatty acids	36.3 mEq./l.
Lipid phosphorus	17.8 mg.%
Protein (globulin)	2.2 gm.%

TABLE 5. THE EFFECT OF DAILY INFUSIONS OF CLEAR ACD HUMAN PLASMA* ON THE PLASMA LIPIDS OF ALLYLAMINE-TREATED DOG NO. 1387—WEIGHT 10.0 KG.

Day	*Plasma injected, ml.*	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*
Control	...	117.6	29.4	8.3	8.5
1	600	166.0	48.5	13.2	9.0
2	725	257.3	75.5	18.0	9.3
3	700	282.0	89.0	23.0	12.6
4	350	297.0	90.0	18.6	16.6
5	600	323.7	102.5	17.5	16.4
6	350	315.0	92.5	13.0	14.5
7	375	323.7	89.5	13.0	14.0
* Pooled clear ACD human plasma used in the above experiment:		156.8	46.9	16.0	12.2

from solution by carbowax, lipids were readily extracted from the protein by ether. Thin frozen sections of the precipitated protein fixed in formalin stained diffusely with sudan dyes. The fresh precipitate dried on a slide gave an intense Schultz reaction for steroids.

Chemical and electrophoretic* analyses of the carbowax-free fraction revealed it to be a mixture of alpha, beta, and gamma globulins. Paper electrophoresis** demon-

* Through the kindness of Dr. J. Haskell Milstone.
** Through the courtesy of Drs. K. Kirkeby and Gerald Klatskin.

strated the contained lipids chiefly in the beta globulin zone, with appreciable quantities migrating with the alpha globulins.

The lipid and protein content of the carbowax-free globulin concentrate given dog No. 1982 appears in Table 4.

Dogs with injuries of their coronary arteries associated with allylamine injections were given repeated intravenous infusions of clear saline solutions of concentrated lipid-rich plasma globulins. The observed effects on the injured segments of the blood vessels were the same with either the crude or more purified fractions. Depending on the condition of the animals or the objective of the particular experiment, there was

TABLE 6. THE EFFECT OF DAILY INFUSIONS OF ACD LACTESCENT HUMAN PLASMA* ON THE PLASMA LIPIDS OF ALLYLAMINE-TREATED DOG NO. 1281. WEIGHT 10.9 KG.

Day	*Plasma injected, ml.*	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*
Control	...	154.0	42.5	12.8	14.5
1	850	315.0	92.5	19.0	17.5
2	600	307.0	85.0	16.0	18.4
3	500	218.0	61.5	13.0	20.5
4	550	191.0	56.0	13.0	20.5
5	200	218.0	70.0	12.0	20.0
6	300	188.0	65.0	12.6	14.4
* Pooled lactescent ACD human plasma used in the above experiment:		186.8	69.0	25.5	14.1

variation in the amounts of protein injected and in the schedules of injections. The results described in this section of the report are based on observations of 17 dogs receiving three daily injections of allylamine followed by 1 to 9 injections of concentrated lipid-rich plasma protein (See Table 10). The animals were sacrificed at varying times after the combined procedures, that is, from one hour after the first injection of globulin to two days after a course of 9 daily injections.

Histological study of the cardiovascular systems of all animals was carried out at the termination of the experiments. Frozen sections stained with sudan IV were prepared for the demonstration of lipids in tissues. A modified Schultz test was utilized for tissue steroids. Paraffin sections were stained with hematoxylin and eosin, Masson's trichrome stain, or Verhoeff's method for elastic tissue. Lipid determinations on the plasma and plasma fractions injected and on the recipient dogs' blood were made using a modified Schoenheimer-Sperry method for cholesterol,[10] a modified Youngburg procedure for lipid phosphorus,[8] and the procedure of Man and Gildea for fatty acids.[7] Nitrogen determinations for estimation of serum proteins were by the usual micro-Kjeldahl procedure.

EXPERIMENTAL RESULTS

The effect of large intravenous injections of human plasma or of lipid-rich plasma protein fractions in dogs.

Dogs tolerated injections of moderate amounts of human plasma well. Massive infusions, above 100 ml./kg., and sometimes less, led to circulatory overload. This limited the amount of human plasma that could be injected into the animals. It was difficult to maintain the dogs' blood lipids, other than lipid phosphorus, above maximal levels[9] for normal humans. A princi-

TABLE 7A. THE EFFECT OF DAILY INFUSIONS OF LIPID-RICH HUMAN PLASMA GLOBULINS* ON THE PLASMA LIPIDS OF ALLYLAMINE-TREATED DOG NO. 1501. WEIGHT 6.8 KG.

Day	*Globulin injected, ml.*	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*
Control	...	107.9	41.3	14.7	13.3
1	300	214.2	84.0	17.0	27.9
2	300	344.4	109.2	21.0	28.5
3	300	294.0	109.2	19.3	27.0
4	300	346.1	116.8	19.3	26.0
5	300	416.7	116.8	27.0	28.0
6	300	416.7	122.6	25.3	26.0
7	300	427.5	151.6	26.6	27.0

* For lipid and protein content of this solution, see Table 2.

pal reason for this limitation was dilution of the injected plasma with the dogs' own blood. It should be emphasized that the plasma of dogs given clear human plasma or clear solutions of human plasma globulin did not become lactescent during the period of the experiment. The effect of intravenous injections of clear or of lactescent human plasma on the blood lipids of dogs is illustrated in Tables 5 and 6.

Exchange transfusions were not attempted. Anaphylactic phenomena in the dogs given human plasma or plasma fractions were only occasionally severe, even with repeated injections. Circulatory overload with massive injections of plasma was the only major technical problem. This was circumvented in other animals by injecting concentrated lipid-rich plasma proteins as described. Large quantities of human plasma globulin were given in this way and the recipients' blood lipids became markedly elevated. Values for the plasma lipids of dogs receiving repeated injections of lipid-rich human globulins are given in Tables 7a and 7b.

Vascular lesions in dogs following allylamine injections and repeated infusions of human plasma.

Eight dogs were given allylamine and clear human plasma, and five animals received allylamine and lactescent human plasma. Protocols of these experiments are summarized in Tables 8 and 9. Necrotizing lesions of the coronary arteries occurred in varying degrees of severity in all but one of these animals. Lipids accumulated rapidly at the sites of arterial injury both in the group receiving clear plasma and in the group receiving lactescent

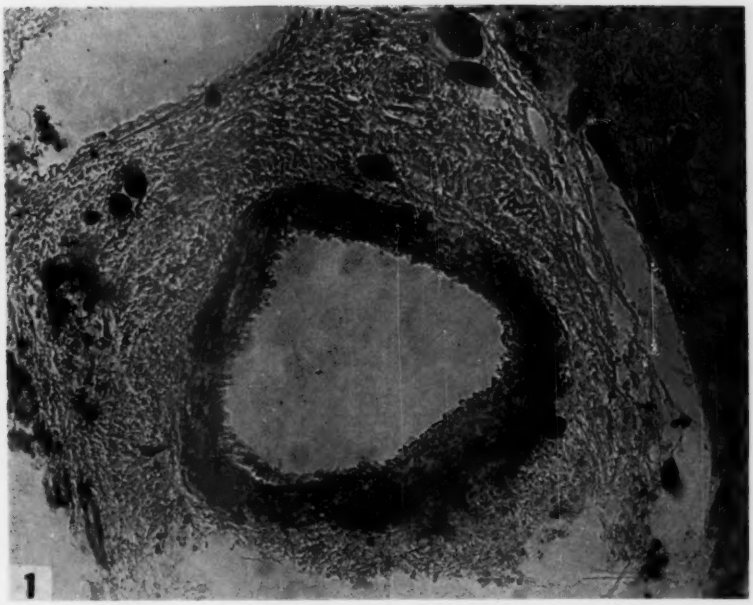
TABLE 7B. VALUES FOR PLASMA LIPIDS AND SERUM PROTEINS OF ALLYLAMINE-TREATED DOG NO. 1982, WEIGHT 6.0 KG., RECEIVING CARBOWAX-FREE HUMAN PLASMA GLOBULIN*

Day	*Globulin injected, ml.*	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Fatty acids mEq./l.*	*Lipid phosphorus mg.%*	*Serum protein Gm.%*
Control	...	139.5	31.5	10.0	10.7	6.06
1	250	....	....	...	...	
2	300	....	....	...	...	
3	250	....	....	...	...	
4	...	666.0	247.8	26.0	25.0	6.19
5	...	....	....	...	...	
6	...	....	....	...	...	
7	...	140.0	46.2	10.0	9.8	5.72

* For lipid and protein content of this preparation, see Table 4.

plasma. Control arterial lesions, associated with injections of allylamine alone, did not stain with sudan dyes. Lipids in the lesions of the experimental animals appeared first as a diffuse medial sudanophilia, detectable within an hour after the first plasma injection. With the passage of time, the sudan-positive material became concentrated into intensely staining globules (Figs. 1 and 2). With lactescent plasma administration, the final accumulation of lipids in the areas of injury was only slightly greater than in the arteries of animals receiving clear human plasma. In both groups, as the lesions became older (5-10 days), phagocytosis of the accumulated lipid droplets was apparent (Fig. 3). In one animal (Dog No. 1281 of the lactescent plasma series) this process became prominent enough to suggest xanthomatous transformation of the intimal proliferation (Fig. 4).

In several animals receiving allylamine and repeated injections of clear or lactescent human plasma, a light diffuse sudanophilia of the inner third of



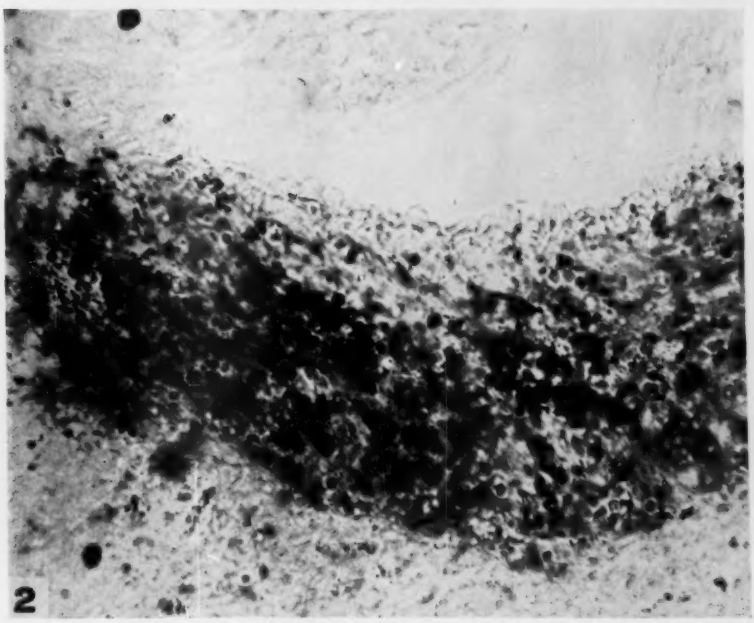
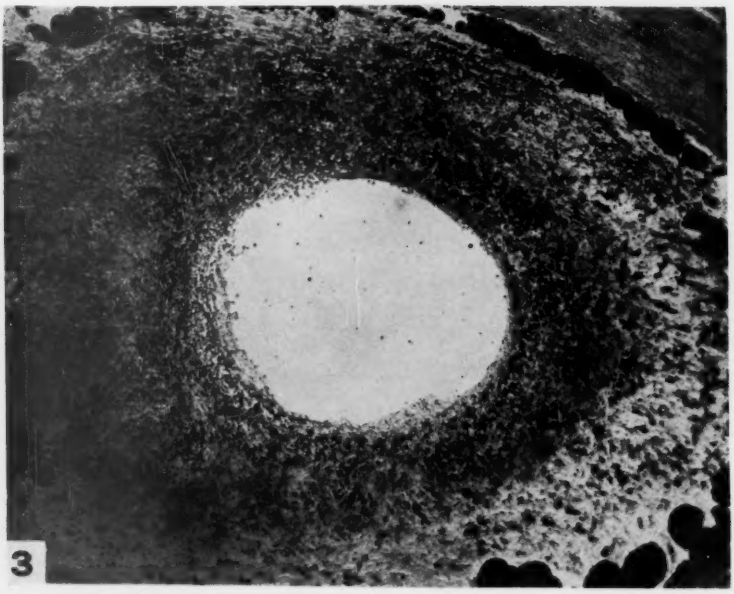


FIG. 1. Coronary artery, dog 1433. Allylamine and lactescent human plasma, three days. Sudan IV stain. The dark masses in the media represent lipids in diffuse and droplet form.

FIG. 2. High power view of segment of a coronary artery of dog 1254. Allylamine and clear human plasma, five days. Sudan IV stain. The dark material in the media is lipid in diffuse and droplet form. Many droplets are within phagocytes.



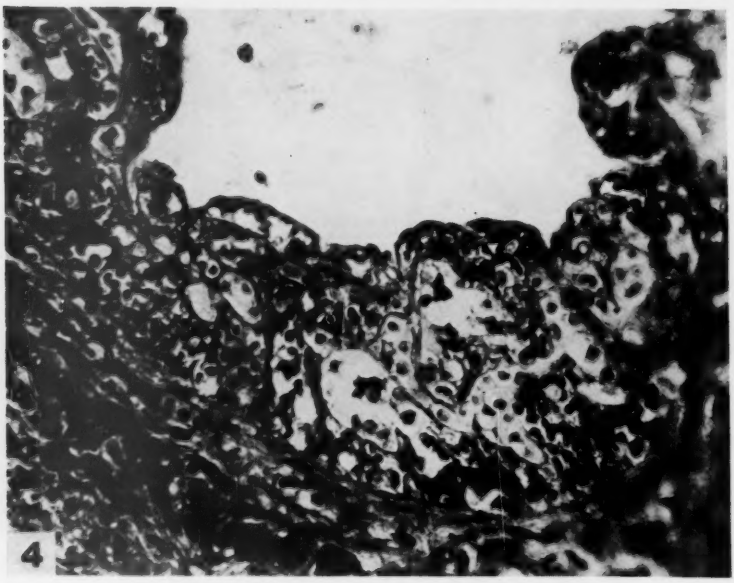
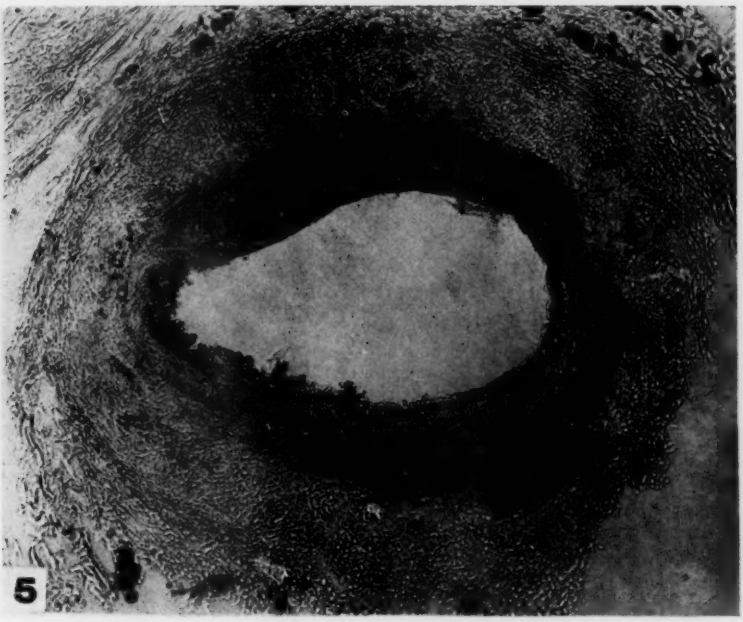


FIG. 3. Coronary artery, dog 1387. Allylamine and clear human plasma, ten days. Sudan IV stain. The dark-staining material in the media and adventitia is phagocytized lipid. Large dark fat cells surround the area.

FIG. 4. High power view of the proliferated intima of a coronary artery of dog 1281. Allylamine and lactescent human plasma, ten days. H & E stain. Accumulations of large, pale lipophages are present in the subendothelial tissue. Red blood cells, lymphocytes and dark masses of "fibrinoid" material are also seen.



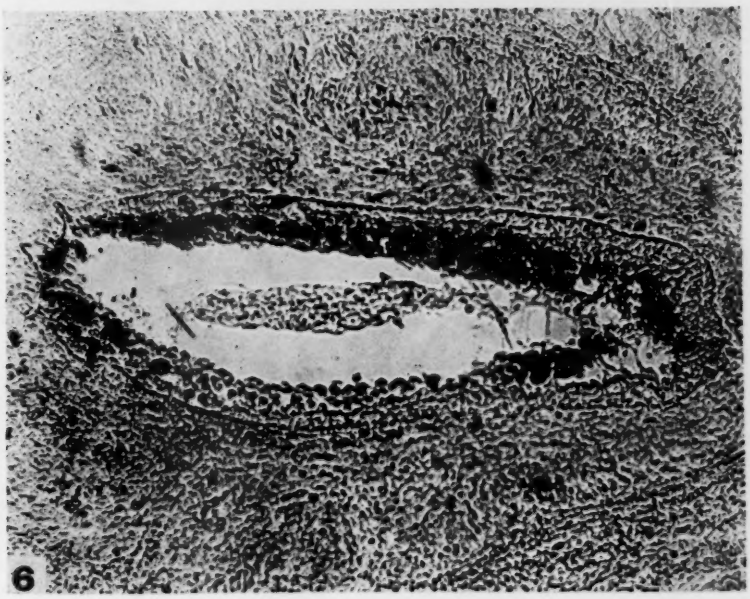
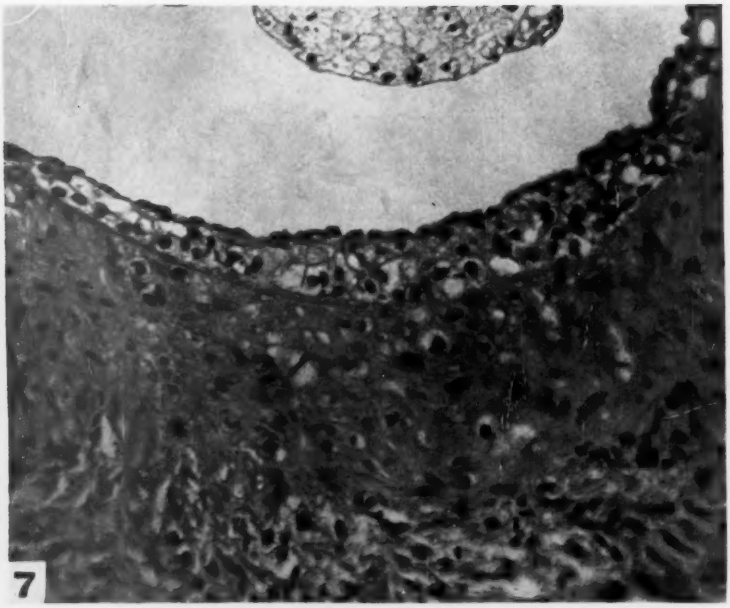


FIG. 5. Coronary artery, dog 1982. Allylamine and lipid-rich human plasma globulin, seven days. Sudan IV stain. The dark areas represent diffuse and phagocytized lipid in the proliferated intima and in the media.

FIG. 6. Coronary artery, dog 1450. Allylamine and lipid-rich human plasma globulin, nine days. Sudan IV stain. Fatty foam-cellular intimal lesion.



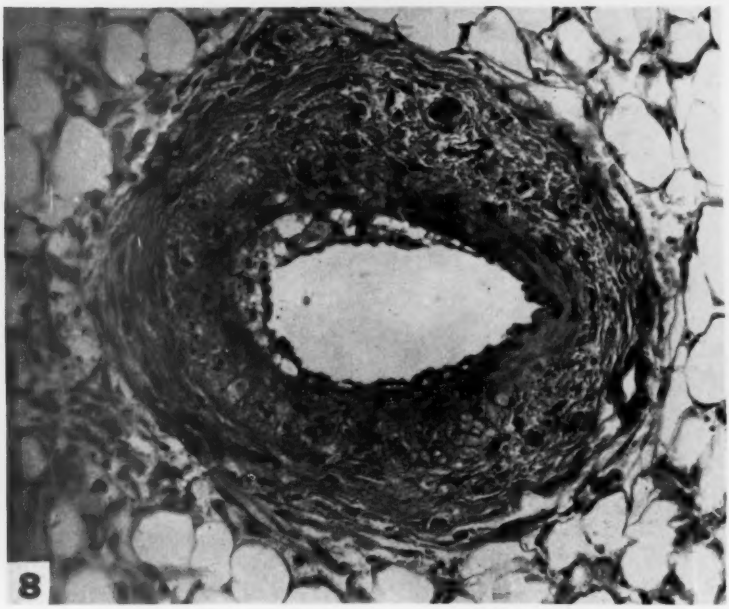


FIG. 7. Segment of coronary artery, dog 1455. Allylamine and lipid-rich human plasma globulin, eight days. H & E stain. Fatty foam-cellular intimal plaque.

FIG. 8. Coronary artery, dog 1457. Allylamine and lipid-rich human plasma globulin, nine days. H & E stain. Crescentic foam-cellular intimal proliferation.

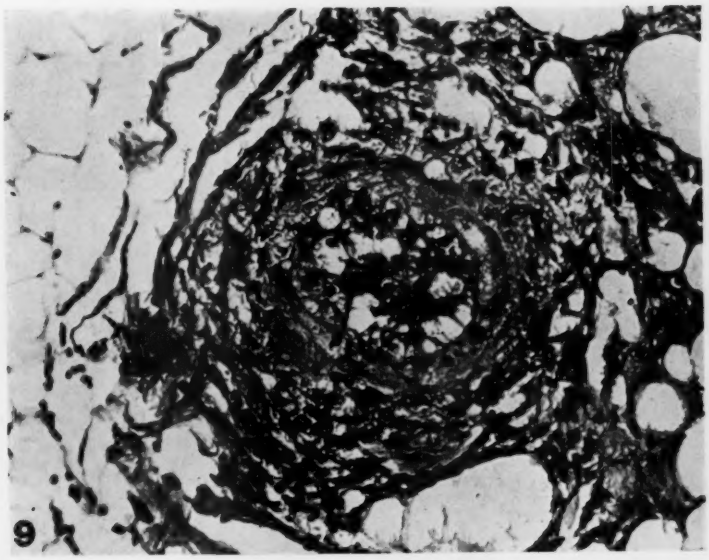


FIG. 9. Small epicardial coronary artery, dog 1982. Allylamine and lipid-rich human plasma globulin, seven days. H & E stain. A fatty granuloma has occluded the lumen.

FIG. 10. Aorta, dog 1982, intimal surface above. Allylamine and lipid-rich human plasma globulin, seven days. H & E stain. The endothelium is raised and there are mononuclear cells and phagocytes in the subendothelial space and in the edematous media.

the aorta, including the intima and inner media, developed. This lesion occasionally exhibited lipid droplet formation and phagocytosis of fatty particles.

TABLE 8. DOGS RECEIVING ALLYLAMINE (15-20 MG./KG., 3 DOSES) AND CLEAR HUMAN PLASMA

Dog No.	*Wt. (kg.)*	*Plasma injections*	*Total plasma injected, ml.*	*Duration of experiment after first plasma injection*		*Vascular lesions*
W 1214	9.6	1	300	30 minutes	S	Medial necrosis of coronary arteries. The injured areas stain diffusely and intensely with sudan IV.
W 1224	8.4	1	500	30 minutes	D	"
W 1232	10.6	1	600	1 hour	S	"
W 1254	12.2	3 in 5 days	700	5 days	S	Medial necrosis of coronary arteries. Lipid in these lesions is in diffuse and droplet form (Fig. 2). Diffuse sudanophilia of inner third of aorta.
W 1255	7.0	5 in 5 days	1150	8 days	S	No allylamine lesions, no lipid deposits.
W 1308	8.2	7 in 7 days	1850	12 days	S	Plasma in lumens of vessels sudanophilic. Much lipid at sites of injury in coronary arteries, in diffuse and droplet form. Scattered lipophages. Lipid in inner third of aorta.
W 1387	10.0	11 in 7 days	3700	10 days	S	" (Fig. 3).
W 1323	10.6	12 in 6 days	5125	12 days	S	"

D = Died, S = Sacrificed.

Lesions of the arteries of dogs after a course of allylamine and a single intravenous injection of concentrated, lipid-rich, human plasma globulin.

Within an hour, the sites of acute arterial injury were diffusely sudanophilic (Table 10). Wherever plasma was observed microscopically in the lumens of vessels, it stained diffusely with sudan IV, in a manner entirely analogous to the staining of the injured mural arterial segments. The lipid staining of these sites was therefore believed to be associated with the entrance of lipid-rich plasma into the vessel walls in areas of increased

permeability. The hearts and blood vessels of dogs were also examined at 2, 6, 24 hours, and four and five days after a single injection of lipid-rich globulin. At these intervals, a progressively increasing number of sudano-

TABLE 9. DOGS RECEIVING ALLYLAMINE (15-20 MG./KG., 3 DOSES) AND LACTESCENT HUMAN PLASMA

Dog No.	*Wt. (kg.)*	*Plasma injections*	*Total plasma injected, ml.*	*Duration of experiment after first plasma injection*		*Vascular lesions*
W 1301	7.2	4 in 4 days	200	8 days	S	Medial necrosis and intimal proliferation of coronary arteries. Slight deposit of lipid in droplet form.
W 1433	8.0	2 in 2 days	900	3 days	S	Medial necrosis of coronary arteries. Deposits of lipid in droplet form and diffusely (Fig. 1).
W 1410	13.0	7 in 4 days	2600	8 days	S	Medial necrosis and intimal proliferation of coronary arteries. Deposits of lipid in droplet form. Scattered lipophages.
W 1281	10.9	11 in 6 days	3000	10 days	S	Medial necrosis, intimal proliferation of coronary arteries; deposits of lipid in droplet form. Many lipophages (Fig. 4).
W 1309	9.5	12 in 6 days	4150	11 days	S	Medial necrosis, intimal proliferation of coronary arteries. Diffuse lipid and lipid in droplet form. Scattered lipophage reaction.

S = Sacrificed.

philic droplets were present at the sites of arterial injury, in a background of diffuse sudanophilia. At the longer time intervals, lipophage activity had already begun.

Changes in the coronary arteries and aortas of dogs following a course of allylamine and repeated injections of lipid-rich human plasma globulin.

At the sites of coronary artery injury in dogs given combined courses of allylamine and of the lipid-rich globulins of human plasma, fatty foam-cellular intimal granulomata were found. Details are given in Table 10. As the animals were sacrificed at varying time intervals, the development of these lesions could be observed. Following the accumulation of sudanophilic

TABLE 10. DOGS RECEIVING ALLYLAMINE (15-20 MG./KG., 3 DOSES) AND LIPID-RICH HUMAN PLASMA GLOBULIN

Dog No.	*Wt. (kg.)*	*Globulin injections*	*Total globulin injected, ml.*	*Duration of experiment after first globulin injection*		*Vascular lesions*
1500	7.0	1	500	1 hour	S	Medial necrosis of coronary arteries; diffuse sudanophilia of injured sites and of plasma in vascular lesions.
1505	6.3	1	600	1 hour	D	"
1597	8.0	1	600	2 hours	S	"
1503	7.0	1	600	6 hours	D	Medial necrosis of coronary arteries; diffuse sudanophilia of injured sites and of plasma. Also beginning lipid droplet formation in areas of injury.
1474	9.5	1	600	24 hours	D	Same as 1503 above, but with many lipid droplets and less diffuse lipid at injured arterial sites. Scattered lipophages.
1438	6.8	1 (crude)	250	3 days	S	No allylamine lesions; no lipid deposits.
1513	8.6	1	600	4 days	S	Medial necrosis of coronary arteries; deposit of fine lipid droplets at sites of arterial injury, mostly extracellular. Scattered lipophages.
1446	7.0	2 (crude)	750	4 days	D	"
1490	9.1	2	800	4 days	S	"
1498	10.0	2	900	4 days	S	"
1514	10.0	1	600	5 days	S	Few medial lesions of coronary arteries with deposited granular lipid; scattered lipophages.
1455	10.0	3	1500	8 days	S	Many foamy sub-intimal and medial lesions of coronary arteries (Fig. 7).

D = Died, S = Sacrificed.

Dog No.	Wt. (kg.)	Globulin injections	Total globulin injected, ml.	Duration of experiment after first globulin injection		Vascular lesions
1501	6.8	7	2100	9 days	S	Many medial lesions of coronary arteries with droplets and irregular masses of lipid. Occasional intimal foam-cell proliferations.
1457	8.0	8	2300	9 days	S	Many foam-cell intimal lesions of coronary arteries where vessels are proliferating; also many fatty medial lesions (Fig. 8).
1450	10.0	7	2920	9 days	S	Medial lesions of several coronary arteries containing droplet lipid; foam-cell intimal lesions of auricular vessels, which have proliferated (Fig. 6).
1338	8.0	9 (crude)	3000	11 days	S	Intimal and medial lipid-containing lesions in coronary arteries. Foam-cell changes occur chiefly in the small left ventricular branches.
1982	6.0	3 (carbowax-free)	800	7 days	S	Massive xanthomatous intimal and medial lesions in coronary arteries. Lipid-containing subendothelial proliferations of aorta (Figs. 5, 9, 10).

S = Sacrificed.

droplets in the area of medial necrosis, lipophages appeared in the media and in the subendothelial space. The accumulation of and the increase in size of these cells led to xanthomatous transformation of the basic intimal allylamine lesion. Changes characterized by phagocytosis were well developed 7-9 days after the first globulin injection and 10-12 days after the beginning of the experiment (Figs. 5, 6, 7, 8, 9). The intimal fatty proliferations often deformed or partially occluded the lumen of the involved artery. The morphological similarity of many of these lesions to the fatty foam-cellular accumulations occurring in arteriosclerosis in man was striking. Uninjured segments of the vessels contained no lipids. In surveying the histological material, it became apparent that the greater the amount of globulin injected or the higher the level of the blood lipids obtained, the more frequent and further developed were the fatty arterial lesions.

After allylamine and repeated injections of plasma globulins, the connective tissue spaces of the inner third of the aortic wall stained diffusely with sudan IV. Occasionally there was evidence of lipid droplet formation, of the accumulation of lipophages, and of subendothelial connective tissue proliferation (Fig. 10).

DISCUSSION

The experiments described in this report indicate that following the injection of clear or of lactescent human plasma or of lipid-rich human plasma globulins into allylamine-treated dogs, there was an accumulation of sudanophilic materials selectively at sites of acute arterial inflammation. After injection of the globulin fractions, xanthomatous transformation of the basic arterial lesions occurred as well. The lipid-connective tissue reactions in the experimental lesions found morphological counterparts in the intimal fatty and foam-cellular changes of arteriosclerosis in man. No lipids were present in *uninjured* segments of the arteries of the experimental animals even in subjects in which values for the blood lipids were greatly elevated during the observation period. This finding stresses the dependence of the intramural lipid accumulations on prior arterial injury.

With injections of clear or of lactescent human plasma, the blood lipid levels of the recipient dogs were not far above the range of high normal for man. The fact that quantities of fatty substances localized in *injured* arterial segments in association with such blood lipid levels is in accord with the observation that in arteriosclerosis the development of lesions is not necessarily dependent upon elevated blood lipid values. However, it was found that the extent of lipid accumulations in the arteries and of xanthomatous transformation of the lesions paralleled the amount of human plasma or of lipid-rich plasma globulin given. The lesions were augmented as the levels of blood lipids in the recipient animals increased. These impressions recalled the well-known occurrence of accelerated vascular disease in persons with hyperlipemic states and also the known facts concerning the establishment of cholesterol atherosclerosis in rabbits.

In dogs receiving lactescent plasma, the respective roles of chylomicrons and of lipoproteins in the deposit of sudanophilic materials in injured arteries could not be determined. However, fatty droplets were also deposited after the injection of chylomicron-free plasma. This suggests the lipoproteins of the injected plasma as the source of these lipids. It is possible that the deposited substances may have originated in the animals' own blood, but this is considered unlikely since in control animals receiving allylamine alone there was no fatty deposit in injured arteries. Also in the

plasma or plasma-globulin injected dogs, lipids appeared in the arteries within 30 minutes to one hour after injection.

Injected as an integral part of their own clear plasma, human lipoproteins were tested for their effects on the basic allylamine lesions in as nearly a natural state as possible. However, it is uncertain whether they would react in dog arterial tissues in a way comparable to native human plasma lipoproteins penetrating the arterial walls of their own hosts. This uncertainty must apply even more forcibly to the plasma globulin fractions injected in concentrated form following separation by chemical means. In spite of these limitations, the appearance of sudanophilic material in the arterial lesions promptly after the intravenous injection of soluble, lipid-rich human globulins suggests that these large molecular substances do localize in foci of arterial injury as do also intravenously injected methylcellulose,[14] India ink,[8] or egg-yolk lipids.[18] Further, the progressive accumulation and phagocytosis of intensely sudanophilic droplets at the injured arterial sites suggests the possibility that water-insoluble fatty particles derived from the original lipoproteins were present in the lesions and stimulated the observed lipophage reactions. An investigation of factors responsible for possible denaturation of lipid-rich plasma proteins locally in the arterial wall is clearly indicated.

The precipitant action of polyethylene glycols on human plasma globulins proved convenient in the preparation of materials utilized in the present experiments.

SUMMARY

Acute inflammatory lesions of the coronary arteries of dogs were established by the intravenous injection of allylamine. Subsequent intravenous injections of chylomicron-free or of lactescent human plasma or of soluble lipid-rich human plasma globulins led to fatty deposits selectively within the basic inflammatory arterial lesions. These accumulations often occurred in association with blood lipid levels in the recipient animals near the range of high normal for man. With increasing elevation of blood lipid values, the lipid deposits in the experimental arterial lesions were augmented. Phagocytosis of the deposited lipids led to foam-cellular transformation that reproduced certain of the morphological sequences of arteriosclerosis in man. The evidence suggests that the injected plasma lipoproteins contributed to the lipid deposits at the sites of arterial injury.

A method for the separation of a lipid-rich globulin fraction of human plasma protein is described. It is based on the precipitation of the globulins by a mixture of polyethylene glycols.

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LYMPHATICS, LYMPH AND LYMPHOID TISSUE. By J. M. Yoffey and F. C. Courtice. Cambridge, Harvard University Press, 1956. 469 pp. $10.00.

In the introductory chapter of this book it is explained that in the lower vertebrates there are lymphatic vessels but no lymph nodes; the lymphoid tissue in fish and amphibia is associated with myeloid tissue in such organs as the intestines, kidney, and liver. Lymph nodes first appear in certain birds, but it is only in mammals that they are invariably present. However, even in mammals, where lymph nodes are always associated with lymphatic vessels, no physiological link has yet been established between the functions of lymph and those of lymphoid tissue. The justification for treating these topics together in a single volume is thus largely anatomical.

In this new edition of *Lymphatics, Lymph and Lymphoid Tissue* Dr. Courtice gives a lucid and authoritative account of the formation and properties of lymph. This involves a discussion of recent views on the structure of capillaries and a detailed account of their permeability to the various constituents of plasma. The striking fact emerges that from 50 to 100 per cent of the plasma proteins pass through the capillaries into the extravascular compartment each day to be returned by the lymphatics to the blood. There are chapters on the physiological significance of the regional lymphatics, including the special role of the intestinal lymphatics in fat absorption, and on conditions involving abnormal function of the lymphatics, notably oedema.

At the time of the first edition of this book in 1941 it was generally agreed that lymph nodes performed three functions: they filtered foreign material from the lymph more or less efficiently, they produced antibodies, and they produced lymphocytes. The problem of the fate of the lymphocyte, posed by the daily entrance of large numbers of lymphocytes into the blood, was unsolved; the function of the lymphocyte was unknown. A careful reading of Professor Yoffey's chapters in the present edition shows that the last 15 years have added little of importance to our understanding of the physiology of lymphoid tissue and the lymphocyte, apart, perhaps, from establishing more firmly that lymph nodes produce antibody. A considerable literature has, of course, accumulated in these intervening years and Professor Yoffey has done a useful service in providing a comprehensive account of it.

J. L. GOWANS

GESTATION. Transactions of the third conference at Princeton, New Jersey, March 6-8, 1956 sponsored by the Josiah Macy, Jr. Foundation. Madison, N. J., Madison Printing Company, 1957. 253 pp. $4.75.

Basic and clinical investigators are now concerning themselves with a critical re-evaluation of many of the old concepts "embalmed" between hard covers of some very old journals, monographs, and treatises. This is certainly an encouraging sign. The present conference concerned itself with eleven topics: (1) maternal endocrine secretions (G. Pincus), (2) maternal hormones in pregnancy (M. X. Zarrow), (3) ovulation cycles and stress (C. P. Richter), (4) secretion of hormones in pregnancy (E. H.

Venning), (5) steroidogenesis in perfused human placentas (G. Pincus), (6) hormones and cellular metabolism (C. A. Villee), (7) gonadal agenesis and sex reversal (E. Witschi), (8) secretory activities of fetal endocrines (A. Jost), (9) hormones and sex differentiation (D. Price), (10) effect of fetal endocrines on fetal growth (L. J. Wells), and (11) interrelations of pituitary functions and pregnancy (R. O. Greep).

Of particular interest to this reviewer were the papers concerned with (a) the effect of hormones on cellular metabolism, (b) secretory activities of fetal endocrines, and (c) interrelations of pituitary functions and pregnancy. Villee's essay introduced a vital spark of interest on the subject of hormones and cellular metabolism. Using C_{14} labeled substrates, and specific enzyme inhibitors, he traced the site of action of estradiol-17β to isocitric dehydrogenase, one of the steps in the tricarboxylic acid cycle, the so-called chief energy-yielding cycle of the cell. It is known that there are eight compounds in the cycle, and energy is released when the hydrogens removed in the several reactions in the cycle pass through the oxidation-reduction enzymes of the cytochrome or electron-transmitter system. This essayist, through a beautifully conceived series of experiments, showed that estradiol affects the enzyme which converts isocitric acid to oxalosuccinic acid. Furthermore, he showed that a DPN-requiring enzyme in the placenta is stimulated by estradiol *in vitro*.

Jost's paper on the secretory activities of fetal endocrines contained an excellent critical appraisal of this complex problem as well as a summary of his researches. Utilizing ten different techniques, Jost showed rather conclusively that the following fetal endocrine glands contain detectable amounts of their hormones:

Glands	*Hormonal Content*
Adrenal medulla	Nor-epinephrine
	Epinephrine
Adrenal cortex	Corticoids
	Progesterone
	Estrogen
Pancreas	Insulin
	Glucagon
Thyroid	Thyroxine
	Triiodothyronine
	Monoiodotyrosine
	Diiodotyrosine
Ovary (late stages)	Estrogens
Testis (late stages)	Androgens
Pituitary	Gonadotropin
	TSH
	Growth hormone
	Diabetogenic factor

In addition to the hormonal content of the fetal endocrine organs, Jost discussed (a) the cytology and histochemistry of these organs, (b) pathological cases, (c) changes after hypophysectomy, (d) sensitivity to "ex-

traneous" hormones, (e) metabolic studies, (f) sensitivity of presumed target organ, (g) effects of removal of certain of the endocrines, (h) effect of hypophysectomy on target organs (related to subject "g"), and (i) graft studies. No student of experimental embryology should be denied the opportunity of reading Jost's essay.

Finally, the essay by Greep on the interrelations of pituitary functions and pregnancy is a very concise and meaningful summary on (a) the ability of intact and hypophysectomized pregnant rats to handle water in the sense of excreting a water load, (b) the differences in weights of fetuses of intact and hypophysectomized rats, and (c) the target organs of the pituitary following hypophysectomy—with special reference to the adrenal glands. Citing data from the Harvard laboratories by several investigators, Greep concluded that in the hypophysectomized pregnant rat there is no substance coming from the conceptus which in any way modified the ability of the animal to excrete a load of water; therefore, this would indicate that nothing from the conceptus is stimulating the maternal adrenal gland to promote a diuretic effect. Greep reviewed several other experiments showing that hypophysectomy of pregnant rats effects a reduction in fetal body weights as well as in placental weight. Also discussed were the experiments of Philip E. Smith which reported that the pituitary could be removed very early from the pregnant monkey without interrupting the pregnancy. The animals remained in a good condition and delivered at term, some delivering normal fetuses. Of additional interest is the fact that the adrenal glands in the hypophysectomized pregnant monkeys remained in a healthy state. In the rat, however, Greer has shown that if the pituitary is removed on the 12th day of pregnancy, then by term the adrenal is reduced in size and is atrophied. Other studies by Knobil and Briggs indicate that the hypophysectomized pregnant animal's adrenal glands are slightly larger than those of the hypophysectomized nonpregnant control animal. It seems that the difference here might be due to some stimulus from the conceptus which partially maintains the maternal adrenal glands. Their incorporation of adrenal ascorbic acid and cholesterol studies support this tenable hypothesis.

Although a small book in size, it is filled with information and will prove most useful among communities of scholars where gestational processes are being investigated.

JOSEPH T. VELARDO

EPILEPSY, GRAND MAL, PETIT MAL CONVULSIONS. By Letitia Fairfield, C.B.E., M.D., D.P.H. New York, Philosophical Library, 1957. 159 pp. $4.75.

This small book on epilepsy has apparently been written mainly for the layman, especially the patient and his family, but it also will be extremely useful for physicians, nurses, psychologists, social workers, and other individuals who work with patients with seizures. There has been a great need for a book such as this, even though there were two attempts over ten years ago by Lennox and Putnam to present this type of information. Within the small number of pages in the book, Dr. Fairfield has included a great deal of information. She begins with an excellent review of the nature of epilepsy

and presents a thorough account of the different types of seizures, their possible causes, how the electroencephalogram is used, and important material concerning the relationship between epilepsy and intelligence. For example, she points out that there may be many different reasons for an apparent association between epilepsy and intellectual defect in some patients, but far from the majority. Also, she presents the different points of view concerning the genetic aspect, including the important data both emphasizing and de-emphasizing this factor. The chapter on treatment is thorough and up-to-date. There is the excellent suggestion that patients carry an identifying card is case of an emergency. One of the really great virtues of this book is an indication of a great sensitivity of approach towards the patient, and this is particularly seen in the chapter concerning the epileptic child. Throughout, Dr. Fairfield presents many of the questions that patients and their relatives and other individuals ask and attempts to answer them thoroughly. Many practical suggestions are given concerning the management of the epileptic child at home and at school. A chapter concerning the employment of epileptic patients indicates in detail the problems to be considered concerning restrictions and types of seizures. Much information concerning training and rehabilitation is given. There are excellent mature accounts of the problems concerning the epileptic in the community, the various schools, hospitals and colonies for epileptic patients, and of the problems concerning epilepsy and the law. The legal problems related to epilepsy are many, for example in relation to driving, marriage, and workmen's compensation, and only in recent years has there been a significant concern about these in this country. The appendix of the book contains an account of the British Epilepsy Association and its activities. The book ends with an excellent series of forty questions and answers related to matters commonly asked about by epileptic patients and their families. There is a small but well-selected bibliography.

This remarkable book is extremely well written and contains a great deal of useful information concerning one of our most important medical problems. It should be required reading for all medical students and all physicians. There might be only one reservation concerning this work. It contains much material relating to the various facilities and local problems concerning the epileptic patients in Great Britain and of course this is a great asset for the British reader. It would be worthwhile to have available a similar analysis of these matters for the United States.

GILBERT H. GLASER

DEXTRAN AND ITS USE IN COLLOIDAL INFUSION SOLUTIONS. By Anders Grönwall. New York, Academic Press, Inc., 1957. 156 pp. $4.00.

There is unquestionably great importance attached to the development of a synthetic plasma volume expander. In the past, several substances have been tried, among them gum arabic, gelatin, and PVP. Over the past 15 years a considerable amount of investigation has taken place concerning the use of dextran. Dextrans are slimy, polysaccharide products of bacterial action upon certain sugars. Such viscous polymers in undegraded state are

unsuitable as colloidal infusion solutions, for which reason they are depolymerized by acid hydrolysis to products of suitable molecular size (average molecular weight 7500).

This book constitutes a review of the history of the development of dextran to its present place, and a review of most of the literature available on the subject. The author, Anders Grönwall, is Professor of Clinical Chemistry at the University of Uppsala, Sweden, and is one of the members of the original Swedish dextran team, whose efforts are largely responsible for the successful establishment of dextran as a clinical tool. In the book, Dr. Grönwall devotes considerable attention to the complex problems of preparation of a dextran with suitable physical properties, and he devotes particular attention to the problem of the relationship between molecular dimensions and desired therapeutic effect. He covers well the available literature on the fate of intravenously administered dextran, and the reports available regarding undesirable side-effects. In all, some 175 references are included in the bibliography. There is little doubt as to the value of a book summarizing this vast literature upon a somewhat controversial subject. One reaches the conclusion that dextran is a well-tried product with a definite place in the medical armamentarium, providing it is used with knowledge of its limitations.

DONALD P. SHEDD

SOME PRINCIPLES OF ENERGETICS IN BIOCHEMICAL REACTIONS. By Irving M. Klotz. New York, Academic Press, Inc., 1957. vii, 64 pp. $3.00.

The increasing emphasis on quantitation in the life sciences requires repeated reference to thermodynamics in discussions of the detailed nature of biochemical reactions. Professor Klotz, in these few pages, sets forth the fundamental laws and applications of classical thermodynamics replacing the Carnot engines and bomb calorimeters with the more familiar phosphate transfer reactions and the sedimentation of macromolecules in an ultracentrifuge.

Using only the most fundamental derivations, the expression for chemical potential or free energy is presented in terms of entropy and energy of the reaction. Subsequent chapters illustrate with biochemical examples how these properties may be employed to predict the feasibility of a reaction and in some measure quantitate the effect of concentration and bond energy on equilibria in enzymatic reactions. In one chapter the basic formulae have been used to derive principles whch define in a limited sense the behavior of matter in such biochemical situations as the ultracentrifuge, osmotic diffusion chambers, and oxidation-reduction systems. The final chapter of the book is devoted to a lucid introduction to the molecular statistical approach to energetics with a brief discussion of quantum theory. Classical entropy is given the mechanical sense of freedom of motion and illustrated in the disorientation of protein denaturation and the deaggregation of metal-protein complex formation.

As stated in the preface, this book is written to give a "reading knowledge" of the terms of energetics to those who desire a very minimum background and perhaps to serve as an introduction to the researcher about

to employ some of these concepts and techniques for the first time. In this latter respect it is unfortunate that very few references to basic research techniques of biochemical application have been included. In essence this book presents expanded definitions of the working quantities of biochemical energetics and their application in a readable form to biologists for whom physics is a distant memory.

R. E. HANDSCHUMACHER

GENETICS IN PLANT BREEDING. Number 9, Brookhaven Symposia in Biology. Associated Universities, Inc., under contract with the U. S. Atomic Energy Commission, 1956. 236 pp. $1.25.

This volume contains the fourteen papers presented formally at a symposium in May, 1956, at the Brookhaven National Laboratories, as well as a record of the discussion, criticism, and less formal contributions which followed the presentation of each paper. The papers, concerned generally with "recent developments in genetic research which are of potential value in plant breeding," may conveniently be grouped as follows: (1) sources of new genetic material, (2) methods in transfer and propagation of genetic material, and (3) statistical and graphic tools for population and genetic analysis.

Three papers discuss the utilization of x-ray-induced mutations in plants of commerical value and the special merits and limitations of this source of mutants. A fourth paper, on the other hand, emphasizes that the possibilities inherent in presently available varieties of food plants and their relatives have not been adequately assayed and urges the preservation in world collections of useful or potentially useful variants. Among the papers concerned with genetic transfer procedures is one describing in detail the transfer of leaf rust resistance from a wild Mediterranean grass to common wheat via several crosses involving polyploids and then an x-ray-induced interstitial translocation. Other papers discuss uses of polyploids, mechanisms of genetic incompatibility, and pollen sterility. The three papers on statistical and graphic procedures describe, respectively, methods of describing genes which exert a small quantitative or continuous effect, methods of testing for gene interactions, and character association analysis, a method of describing simultaneously the variations in a number of characteristics in many members of a population.

An interesting paper by workers at the University of Wisconsin described x-ray-induced biochemical mutants of the ascomycete causing apple scab, and presented data indicating a correlation between loss of pathogenicity and genetic loss of ability to synthesize certain essential amino acids. An independent project investigates the fundamental genetics of apple, with the practical aim of developing resistant strains. However, information is exchanged and the apple project provides differentially susceptible media for establishing physiological races of the fungus. In the words of the authors: "The work with apple scab furnishes an example of the happy union that can exist between basic and applied aspects of research. The fundamental studies guide the breeding program, which in return furnishes materials and results of great value to the basic research."

It is because of the increasing cooperation of the science of genetics and the art of plant breeding and the rewards accruing to both disciplines that this volume should be of interest, if not indispensable, to both the geneticist and the plant breeder.

BROOKE B. WEBBER

PROGRESS IN NUCLEAR ENERGY, Series Seven, Medical Science, Volume One. Edited by J. C. Bugher, J. Coursaget, and J. F. Loutit. New York, McGraw-Hill Book Co., Inc., London, Pergamon Press Ltd., 1956. 165 pp. $6.00.

Nuclear energy has firmly established itself as a separate branch of physical science, engineering, and medicine. This new publication, *Progress in Nuclear Energy,* consists of eight series, namely: I. Physics and Mathematics, II. Reactors, III. Process Chemistry, IV. Technology and Engineering, V. Metallurgy and Fuels, VI. Biological Sciences, VII. Medical Sciences, VIII. Economics of Nuclear Power. The editors aimed to encompass the progress achieved in all facets of nuclear energy and make it easier for the scientists and engineers to obtain information outside their special field.

The Geneva Conference on Peaceful Uses of Atomic Energy in 1955 provided for the first time the free flow of information on nuclear energy between the scientists of all nations. The first volume in each series was published as soon as it was technically feasible after the Geneva Conference.

This volume, *Medical Sciences,* consists of many critical review articles on significant topics discussed and also a few of the actual papers presented at the Geneva Conference. The first two chapters review the use of radioisotopes in medical diagnosis and their significant contribution to metabolic study of the human body. The third chapter deals with treatment of diseases by external radiation sources, such as radiocobalt, radioiridium and radiocaesium, and supervoltage devices. It was reported by Russian scientists that 160 oncological establishments in the Soviet Union had almost simultaneously been supplied with sets of radioactive cobalt preparations in the shape of small tubes and needles of various sizes containing from 0.5 mg. to 35 mg. radium equivalents. Apparently, the telecobalt units used in Russia contain much smaller amounts of active source than the units installed in this country and Canada (as below 500 curies vs. 1,000-1,500 curies).

The fourth chapter deals with radioisotope for internal therapy. Long-term observation and results obtained with the use of P^{32}, I^{131}, and radiocolloids were critically reviewed. The hope of finding a method of selectively localizing a radioisotope in cancer cells in the human body has not yet been realized.

The fifth and sixth chapters deals with principles and standards of radiation safety and health and safety in reactor and chemical processing plants. Attention was confined to two main groups: (1) Maximum permissible exposure standards, (2) Administrative and legal problems. In the U.S.A., as in many other countries, the trend is toward legislative regulations in controlling radiation hazard, particularly regarding industrial

applications of radioactive material. In Soviet Russia, it was reported that when the average dose received by a worker exceeds 10 times the natural background level, he is granted a shorter working day and a longer annual holiday. A six-hour day is operative in the case of work with nuclear reactors, with gamma ray radiography of metal and with radioactive luminous compound, etc. In the case of gamma ray therapeutic work in hospitals, the working day is reduced to five hours.

The last chapter, Dosimetry of Ionizing Radiation, is of primary importance. The article deals primarily with experimental determination of absorbed dose of radiation by ionization measurements. The Bragg-Gray relation is discussed in detail to indicate the conditions under which the measurements are to be made. Some other methods based on calorimetric measurements or determination of chemical changes are also reviewed.

While no startlingly new medical discoveries had been concealed until the Geneva Conference, there is a definite need for a critical summary, such as this volume represents, of the great amount of scientific material that was presented by various countries at the Geneva Conference. Each chapter begins with an abstract and the historical background is included the first time a particular subject is discussed. The bibliography is extensive in some chapters. It should prove useful to radiologists and other scientists who would like brief but accurate information on recent progress in nuclear energy in medicine.

C. H. CHANG

SCHOOL HEALTH AND HEALTH EDUCATION. By C. E. Turner, D.P.H., C. Morley Sellery, M.D., and Sara Louise Smith, Ed.D. 3d ed. St. Louis, C. V. Mosby Company, 1957. 466 pp. $4.00.

This volume is a bargain for teachers and school health personnel, for whom it was prepared primarily, but it will be useful for physicians, dentists, nurses, and others who work with children, and their parents. Medical students and graduate students will find suggestions presented briefly and clearly. Prepared by outstanding leaders with practical experience in health, medical service, education, and the administration of school health programs, and brought up to date to reflect ideas of useful school health policies and procedures, the book is filled with helpful information and references.

New concepts, tested procedures, and evaluation methods are contained in the twenty chapters divided into two parts: the *basis* for school health and health education, and school health and health education *in action*. The appendix contains specific health education objectives and first aid procedures.

In a chapter on the school health team, for example, attention is given to an effective teaching staff and to maintaining the health of the staff in reference to such factors as: problems of occupational hygiene, health examinations, communicable disease control, and the health aspects of administrative policies. Then are discussed duties of members of the school health team, including the classroom teacher, the school administrator, the physician serving the school, the nurse, the physical educator, the dentist, the nutri-

tionist, the dental hygienist, the health coordinator, the health chairman in the elementary school, and the school custodian.

Consideration is given to health examinations in the physician's private office, and to the roles of the parent, nurse, and teacher. Chapters deal with the promotion of mental health, with family life and sex education, and with the much neglected subject of evaluation. Rarely can one write as favorable a review with such a clear conscience!

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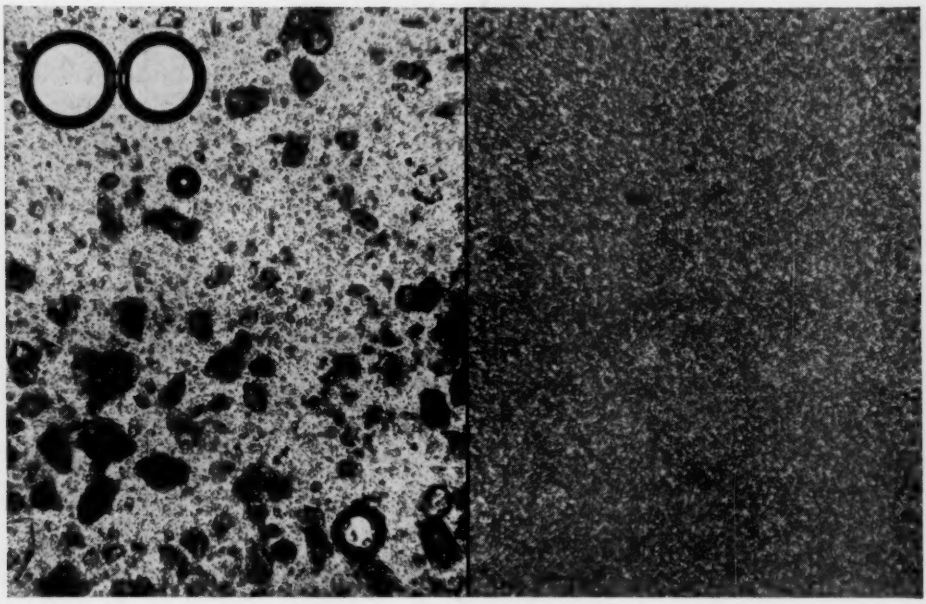

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